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THE CHEMICAL INVESTIGATION OF SOME GRAMINEAE OILS

By

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Introduction

Although a large number of reports on the chemical studies of rice and barley, the important foodstuffs in this country, have already been made, there have been comparatively few reports on millet, (*Panicum miliaceum* L.), Japanese barnyard millet (*Echinochloa crusgalli* BEAUV. subsp. *edulis* HONDA var. *typica* HONDA) and Italian millet (*Setalia italica* KUNTH.) which rank with rice and barley as foodstuffs. As already reported[9] in the results of physico-chemical studies on proteins of the above mentioned three kinds of millets, the author had been engaged in the chemical investigation of these foodstuffs and his attention was then directed to the study of the oils of millet and Japanese barnyard millet in order to obtain further knowledge of them.

In 1887, KASSNER[10, 11] investigated millet oil and found ricinoleic, stearic, and millet oil acid ($C_{18}H_{32}O_2$) as fatty acids. Since then ANDREJEW[7] has also studied the same subject and recorded that erucic, oleic, ricinoleic and linolic acid are found in the saponifiable part of the oil. More recently the oil was investigated by DUNBAR and BINNEWIES[8], who reported that millet oil gave palmitic, oleic, linolic, iso-linolic, carnaubic and daturic acid through saponification.

On the other hand, an unsaponifiable substance was isolated by KASSNER[10, 12, 13] and DUNBAR and BINNEWIES[8] from millet oil.

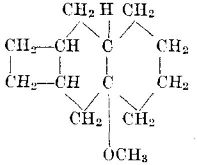
The former collected the crystalline substance which crystallised out on the bottom of the vessel when millet oil was left in a cold dark room, and designated it "panicole", melting at 285° (not corrected). It was stated to be optically inactive and to have the formula $C_{13}H_{10}O$. Under the action of hydroiodic acid in a sealed tube at 150°, methyl iodide was produced. By similar method, methyl chloride was obtained along with a solid substance which melted at 78° but could not be obtained in a crystalline form. The analysis gave the composition, $C_{12}H_{18}O$ for the latter.

On the oxidation of panicole with bromine and chromic acid in glacial acetic acid KASSNER obtained the products, $C_{13}H_{20}O_2$ and $C_{13}H_{18}O_5$ respectively. It was stated that the latter compound, panicolic acid, was a dibasic acid and has the formula,

$$C_{10}H_{13}\begin{cases} OCH_3 \\ COOH \\ COOH \end{cases}.$$

Panicolic acid gave at 120–130° an acid anhydride, melting at 190°.

By the pyrogenetic treatment of panicole, hydrogen, acetylene and methane were obtained, and panicole was considered to be a hydrogenated naphthalene derivative, having the constitutional formula of



DUNBAR and BINNEWIES[8)] recognised the pearly white crystals, m. p. 279° (corrected) gradually deposited from the oil extracted from the proso millet (*Panicum miliacum*) by ether or light petroleum. As this substance could not be identified, it was called "prosol". Prosol has the formula $C_{24}H_{36}O_2$ and gave negative tests for aldehyde, acid, carbohydrates, phenol, ester, anhydride, lactone or ketone groupings, but a positive acetylation for an alcoholic grouping. With hydroxylamine it produced an unsatisfactory quantity of a precipitate, presumably an oxime.

They concluded that prosol was an alcohol-ketone which was, in several respects, allied to the phytosterols.

The author has separated oleic, linolic, iso-linolic acid as unsaturated and palimitic, carnaubic acid as saturated from the saponifiable matters of millet oil. The carnaubic acid (m. p. 115.5–116.5° not corrected) was identified with its tribromoanilide, having a bromine content of 34.64 per cent. He has isolated a substance melting at 282–283° (not corrected) which corresponds to KASSNER's "panicole" or the "prosol" of DUNBAR and BINNEWIES. As a result of the close investigation of this substance, however, it has been proved to be quite different from "panicole" or "prosol", and the substance was named "miliacin" for which the formula $C_{32}H_{54}O$ has been assigned. According to the analytical results obtained miliacin is identical with sycoceryl alcohol ($C_{32}H_{54}O$) obtained by hydrolysis from sycoceryl acetate (named by DU LA RUE and MÜLLER in 1854) which was isolated from the resin of Fucus rubignosa by Edward H. RENNIE.[10)] Yet these two substances seem to be quite different, since the melting point of sycoceryl alcohol, 114°, is much lower than that of miliacin. Miliacin has $[\alpha]_D^{25^\circ} = +20.2^\circ$ and the LIEBERMANN-BURCHARD's reaction of miliacin is slightly different from

that of phytosterol, for the former assumes a red violet which rapidly deepens to wine-red, while the latter shows a green colour. On treating miliacin with acetyl chloride in a sealed tube at 150° for three hours, white crystals are obtained from which, after several fractional recrystallisations from alcohol, two substances are isolated. One of them melts at 80–87° and the other at 112–114°.

The former compound was not further studied owing to the difficulty in obtaining it in a pure condition. It has no sharp melting point. The latter was, however, investigated and was shown to be a hydrocarbon of the composition $(H_5H_8)_6$ by analysis and molecular weight determination. These results appear to be interesting in that miliacin is closely related to isoprene which is considered to be genetically the nucleus of terpene. Therefore miliacin may be considered to bear a close relation to the terpenes. In order to examine accurately the function of oxygen in the miliacin molecule, first of all the methoxyl group was examined, with negative results. Acetyl chloride, anhydrous acetic acid, benzoyl chloride and thionyl chloride, were used under various conditions while in each case the unchanged original substance was recovered, showing it to have no hydroxyl group contrary to phytosterols.

On treating miliacin with phosphorus pentachloride, two kinds of chloride are secured, one of which crystallises out from alcohol, in rhombic hexahedrons and melts with decomposition at 238°, getting dark brown at 230°; the other melting at 220–222°, crystallising in prism form from alcohol. On analysis it was shown that these two products contain oxygen and have the compositions $C_{32}H_{51}Cl_3O$ and $C_{32}H_{53}ClO$ respectively. Accordingly the oxygen in miliacin could not be in a hydroxyl group but should be considered as an ether-shaped one.

Catalytic hydrogenation, platinum black or platinum oxide being used as a catalyser, showed that miliacin seems to be a saturated compound. By zinc dust distillation a liquid product was obtained together with a resinous substance having an aromatic odour. Although the yellowish liquid portion is likely to be a sesquiterpene, further investigation of the compound is still in progress.

The absorption spectra of miliacin were examined and general absorption found.

From unsaponifiable matters, a phytosterol was also separated, melting at 135–136° (not corrected) and having $[\alpha]_D^{25°} = -31.2°$, its acetate melting at 128° and having $[\alpha]_D^{25°} = -38.1°$. The absorption

bands of phytosterol were examined and this phytosterol found to contain a small amount of ergosterol. Another phytosterol melting at 130–133° was also isolated; it cannot, however, be regarded as a pure substance and its recrystallisation from alcohol is difficult. As to phytosterol, ANDERSON and his collaborator[1,2,3,4,5,6,15] have found various kinds of phytosterols with different melting points and optical rotatory powers, from plant oils. HEINRICH WIELAND and MITIZO ASANO[19] have obtained various kinds of sterols by preparing sterol benzoate from yeast. From these facts, the substance melting at 130–133° is considered to be a mixture of several kinds of sterols. *Syntherisma sanguinalis* DULAC var. *ciliaris* HONDA, '*mehishiba*', is closely related to ***Panicum miliaceum*** L. from the botanical standpoint and was formerly classified in the same genera. The components of the oil of the former were also studied with nearly the same results as in the case of the latter, obtaining palmitic, oleic, linolic and carnaubic acid, miliacin and phytosterols.

Next, the author has investigated Japanese barnyard millet oil in the same way as in the case of millet oil with the following results. He has isolated only palmitic acid as a saturated fatty acid, and, as unsaturated ones, oleic, linolic and iso-linolic acids from the saponifiable matters. Moreover, from the unsaponifiable matters the substance melting at 280°, which is near the melting point of miliacin (m. p. 282–283°), has been isolated. But when mixed with a specimen of miliacin it melts at about 250°, which shows clearly that it differs from miliacin. The author thereupon proposes to name it "crusgallin" to distinguish it from miliacin. From the results of the analysis and the determination of molecular weight, the formula $C_{32}H_{54}O$ has been assigned to crusgallin. Judging from the molecular formula crusgallin should be considered as an isomer of miliacin. The general properties of crusgallin are similar to those of miliacin. Crusgallin has $[\alpha]_D^{20°} = +16.3°$, and is not effected by the hydrogenation procedure, using platinum black or platinum oxide as a catalyser, and shows a general absorption on the examination of absorption spectra. Crusgallin belongs to the monoclinic system and has perhaps a hollohedral symmetry in crystallographical observations as shown in the figure, measurement of which has been carried out by Mr. T. SHIGA, to whom the author expresses his hearty thanks. Further, from the unsaponifiable matters, the substance sparingly soluble in 95 per cent. alcohol was isolated, sintering at 62°, melting at 63° in white turbidity, and at 83° clearly. This substance, in spite of having a sterol colour reaction, seems to be allied to wax.

The purification of this substance is so difficult that the pure product could not be obtained. Its specific rotatory power is +1.8 at 25° in chloroform. Moreover, from the portion easily soluble in 95 per cent. alcohol a phytosterol, melting at 136.5–137.5°, was isolated and had $[\alpha]_D^{25°} = -21.3°$. Its acetate melted at 124–126° and has $[\alpha]_D^{27°} = -26.0°$. When a phytosterol of Japanese barnyard millet is mixed with that of millet, it melts at 136° without showing any depression. The absorption spectra of this phytosterol were examined and found to contain a small amount of ergosterol. From the portion extremely soluble in 95 per cent. alcohol, a substance melting at 91.5° (not corrected) was separated in needles, having $[\alpha]_D^{27°} = +61.9°$. From the results of the analysis and the determination of the molecular weight of this substance, crusgallin ($C_{32}H_{54}O$) may be considered as an isomeride because the difference of numerical values between the results falls within experimental error. The value of this substance, however, is also close to that of $C_{30}H_{50}O$ and $C_{29}H_{48}O$. Hence to which molecular formula this substance may be assigned will be determined by further investigation. This substance is named "hiyeol", for the sake of convenience. The absorption spectra of hiyeol were examined and general absorption found, as in the former cases.

Summarising the results of the present investigation, millet (*Panicum miliaceum* L.) oil and *'mehishiba'* (*Syntherisma sanguinalis* Dulac var *ciliaris* Honda) oil are essentially composed of glycerides of the following fatty acids: oleic, linolic, iso-linolic, palmitic and carnaubic. From unsaponifiable matters, in addition to the presence of phytosterol (m. p. 135–136°), a white crystalline substance is obtained and designated "miliacin" ($C_{32}H_{54}O$) which is regarded as a new compound bearing a close relation to terpenes.

Next, Japanese barnyard millet (*Echinochloa crusgalli* Beauv. subsp. *edulis* Honda var. *typica* Honda) oil is composed chiefly of glycerides of the following fatty acids: oleic, linolic, iso-linolic and palmitic. From unsaponifiable matters, besides a phytosterol (m. p. 136.5–137.5°), two new compounds are obtained which melt at 280° and at 91.5° respectively. To the former, crusgallin ($C_{32}H_{54}O$) is designated and to the latter hiyeol.

As above described, the author has isolated fatty acids from saponifiable matters and has also obtained sterols, miliacin, crusgallin and hiyeol from the unsaponifiable part of the oils of millet, Japanese barnyard millet and *'mehishiba'*. Furthermore, it is worthy of note

that the oils of these plants contain unsaponifiable matters besides phytosterols, which are identical or closely related substances. Miliacin is obtained from millet as well as '*mchishiba*', whilst crusgallin from Japanese barnyard millet and these compounds have exactly the same molecular formula $C_{32}H_{54}O$, but are different. The author has shown that these special unsaponifiable matters are different from panicole or prosol described in the literature and has suggested that these are genetically closely related to the terpenes.

Experimental

CHAPTER I

On the Oil of *Panicum miliaceum* L. (MILLET)

The material employed in this investigation was produced in the suburbs of Nagoya the preceding year, and was ground by means of a stone mill. 133.8 kilograms of ground material (moisture 13.0 per cent), on extraction by continuous percolation with hot benzine, yielded 5.2 kilograms of oil.

Some constants of the natural oil were determined, with the following results:

Specific gravity (15°)	0.933
Refractive index (30°)	1.467
Acid value	72.93
Saponification value	170.65
Iodine value	120.68
Hehner value	91.44
Reichert-Meissl value	1.10

The contents of products insoluble in acetone, unsaponifiable matters, and solid and liquid fatty acids in the oil were all determined by the following method:

On treating one part of the oil with about twenty parts of acetone a precipitate was produced, which was collected, dried in a vacuum over sulphuric acid, and weighed. From the portion soluble in acetone, the contents of unsaponifiable matters were estimated by BÖMER's method; and the weight of unsaponifiable matters insoluble in 95 per cent. alcohol was also determined. This unsaponifiable matter and the part insoluble in acetone are crude miliacin. The contents of the solid and liquid fatty acids in the residual portion, after the unsaponifiable matters had been removed, were determined by the lead-soap-ether method. The analytical results were as follows:

	I	II
Subst. g.	18.00	15.41
Precipitate by acetone g.	0.3472 (1.93%)	0.2794 (1.81%)
Unsaponifiable matters g.	1.429 (7.92%)	1.118 (7.26%)
95% alcohol insoluble unsaponifiable matters g.	0.0377 (0.21%)	0.0319 (0.21%)
Solid fatty acid g.	1.719 (9.53%)	1.488 (9.84%)
Liquid fatty acid g.	13.22 (73.44%)	11.37 (73.75%)

Some constants of solid and liquid fatty acids thus obtained were measured with the following results.

Substances		Refractive index at 30°	Iodine value	Neutralisation value
Solid fatty acids	I.	—	16.90	209.59
	II.	—	18.29	207.81
Liquid fatty acids	I.	1.4552	145.99	196.38
	II.	1.4590	144.05	198.63

Separation of Miliacin and Phytosterol

One hundred gram batches of the oil and 200 c.c. of alcoholic potassium hydroxide solution, which was previously prepared by mixing 60 c.c. of potassium hydroxide solution, made by dissolving 200 grams of potassium hydroxide in water, the whole being made up to 300 c.c., and 140 c.c. of 95 per cent. alcohol, were put into a one litre flask. The contents of the flask were completely saponified on the boiling water bath for about an hour under the reflux-condenser, and were treated with 300 c.c. of water. This was introduced into a 2 litle separatory funnel containing 300 c.c. of water. In order to extract the unsaponifiable matters 800 c.c. of ether was poured into the separating funnel, which was shaken vigorously for about an hour. On allowing to stand for about half an hour, a clear sharp separation took place and the upper ethereal layer was separated from the lower. This extraction process was repeated until the upper ethereal layer did not finally assume a yellow colour.

In the course of this extraction, at the first treatment about 500 c.c. of ethereal solution were obtained, while at the succeeding treatment about 800 c.c. of the solution were secured.

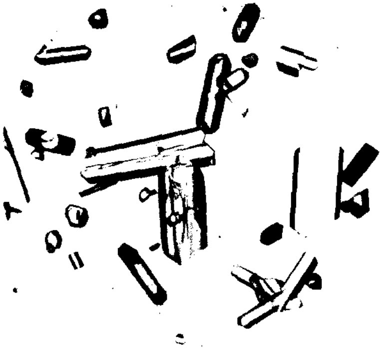
On distilling off the ether from the solution which contained alcohol used for the saponification, a considerable amount of scaly crystals separated. These were collected while hot. The crystalline mass thus obtained was crude miliacin.

After keeping the filtrate in a cold dark room for about a week, a

Properties of Miliacin

Miliacin, crystallises in hexagonal plates as is shown in the figure, is soluble in ether, chloroform, carbontetrachloride, xylene, benzene, sparingly soluble in methyl and ethyl alcohol, anhydrous acetic acid, ethyl acetate, acetone and insoluble in cold alcohol or water. Its melting point is 282–283° (not corrected).

Fig. 1



miliacin

Colour reaction:—About 5 miligrams of the substance were dissolved in 2 c.c. of chloroform, and 2 c.c. of conc. sulphuric acid were added slowly to the solution. The layer of chloroform was immediately represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. But after a short time *the layer of chloroform turned to a reddish colour.* (HAEGER-SALKOWSKY).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added gently. The layer of the anhydrous acetic acid assumed a red-violet which after a short time changed into a wine-red, while the layer of sulphuric acid showed no colour. (LIEBERMANN-BURCHARD).

great deal of the yellow crystalline mass was produced, which was separated and dried in a vacuum desiccator. This was treated with 95 per cent. alcohol and roughly separated into three parts; those readily soluble, those with difficulty soluble, and those insoluble in alcohol. In this case the former two parts consisted chiefly of sterols but the third part of miliacin.

The crude miliacin thus obtained was recrystallised from benzene and finally from xylene and was separated in colourless hexagonal plates, melting at 282–283° (not corrected).

The crude phytosterol being the part readily soluble in alcohol was recrystallised several times from 95 per cent. alcohol and colourless crystals were obtained which melted at 135–136° (not corrected). It was, however, so difficult to obtain a phytosterol in a pure condition from the part with difficulty soluble in alcohol that after several recrystallisations from alcohol the substance melted still indefinitely at 130–133° (not corrected). This gave a marked LIEBERMANN-BURCHARD reaction.

Another Method of Separating Miliacin from Phytosterol

This separation method was based largely upon the fact that miliacin is with difficulty soluble in cold acetone, while the oil and phytosterols are soluble in the solvent.

One hundred gram batches of the oil were introduced into about 1,000 grams of acetone, stirred for a while, and kept standing in the ice-chest for about 3 hours. The brown precipitate which settled on the bottom of the vessel was filtered and dried. This product was extracted with petroleum ether (b.p. 40–46°), using a Soxhlet apparatus for more than one week. After recovering petroleum ether, miliacin was crystallised out in colourless plates which, after several recrystallisations from benzene and then xylene, melted at 282–283° (not corrected) and was proved to be identical with the substance obtained by the above hydrolytic method, because there was no depression of the melting point when they were mixed.

The oil, after miliacin had been mostly removed, was treated with alcoholic potash. Phytosterol was obtained and a small amount of miliacin.

In the first and the second isolation method, 3.9 kilograms of the oil had been used and 33 grams of the purest miliacin obtained, or 0.7 per cent. of the original oil. The total yield of the purest physosterol (m.p. 135–136°) was 5 grams or 0.13 per cent. of the original oil.

Specific Rotatory Power

The specific rotatory power of the substance was determined with the following result. (chloroform as a solvent).

$$[\alpha]_D^{25^\circ} = \frac{+1.32^\circ \times 100}{2 \times 3.262} = +20.2^\circ$$

Analysis

I.	Subst. 4.996 mg.,	CO_2 15,845 mg.,	H_2O 5.32 mg.,	C 84.53;	H 11.92%
II.	„ 5.050 „	„ 15.650 „	„ 5.41 „	„ 84.52;	„ 11.99 „
III.	„ 4.588 „	„ 14.230 „	„ 4.851 „	„ 84.59;	„ 11.83 „
IV.	„ 4.606 „	„ 14.255 „	„ 4.945 „	„ 84.41;	„ 12.01 „
V.	„ 3.826 „	„ 11.880 „	„ 4.100 „	„ 84.68;	„ 11.99 „
VI.	„ 4.105 „	„ 12.729 „	„ 4.427 „	„ 84.57;	„ 12.07 „
VII.	„ 0.1236 g.	„ 0.3840 g.	„ 0.1340 g.	„ 84.73;	„ 12.13 „
			Average	„ 84.57;	„ 11.98 „
			$C_{32}H_{54}O$ requires	„ 84.50;	„ 11.99 „

Molecular Weight

The determination of the molecular weight was carried out according to RAST's micro-method and the cryoscopic method, using benzene as a solvent, with the following results:

1. RAST's method.

Camphor 0.15 g., subst. 0.015 g., $\Delta t = 9^\circ$,

$$M = \frac{40 \times 100}{9} = 444.$$

$C_{32}H_{54}O$ requires 454.

2. Cryoscopic method

Subst. 0.05 g. in 19.45 g. benzene,
Depression of the freezing point: 0.030°,

$$M = \frac{5.12 \times 2.57}{0.030} = 439.$$

$C_{32}H_{54}O$ requires 454.

Catalytic Hydrogenation

Catalytic hydrogenation was carried out, using a mixture of 0.2701 gram of the substance, 15 c.c. of hexane and 0.1 gram of platinum black for about 24 hours, without revealing absorption of hydrogen. The recovered substance, after recrystallisation from benzene, melted at 282–

283° (not corrected) as in the case of the original substance. It was further mixed with the original substance, without depression of the melting point ensuing. The same treatment was tried, using platinum oxide as a catalyser, but the hydrogenation of miliacin did not take place.

Treatment with Benzoylchloride

A small amount of miliacin was dissolved in pure pyridine and a calculated amount of benzoylchloride added. The mixture was stirred for a while and kept in a cold room overnight. This was then introduced into a large amount of water and the resulting compound crystallised from benzene, when it separated in colourless plates, melting at 282–283° (not corrected). On mixing with the original substance no change in melting point was observed.

Treatment with Anhydrous Acetic Acid

One gram of the substance and 10 c.c. of anhydrous acetic acid were introduced into a tube and were heated at 150° for three hours after sealing the tube. Then the contents of the tube were introduced into cold water and crystallised from benzene. In this procedure though a small amount of the original substance was changed into a resinous matter, a large amount of it was not changed, since the depression of the melting point was not observed when it was mixed with the original product.

Treatment with Acetylchloride

Five grams of the substance and 50 c.c. of acetylchloride were introduced into a tube and heated at 150° for three hours after sealing the tube, when the contents of the tube were assumed to be rosetted. A small amount of unchangeable matter remaining in the tube was filtered, and from the filtrate excess of acetylchloride was removed under reduced pressure; 0.08 gram of unchanged substance, 1.5 grams of resinous matter and 2.92 grams of white plumelet-like crystals were subsequently obtained. On recrystallisation of these crystals, two needle-like substances, melting at 80–87° and at 112–114°, were separated. The former substance (m.p. 80–87°) was not considered to be pure. The latter (m.p. 112–114°) was analysed and proved to be a hydrocarbon as follows:

Subst. 4.140 mg., CO_2 13.417 mg., H_2O 4.385 mg.;
,, 3.989 ,, , ,, 12.927 ,, , ,, 0.257 ,, ;

C 88.39, 88.38; H 11.85, 11.94%

$(C_5H_8)_n$ requires C 88.16, H 11.85%.

The molecular weight of the product analysed above was determined by the cryoscopic method with the following results. (benzene as a *solvent*).

Subst. 0.0818 g., benzene 17.43 g.

Depression of the freezing point (Δt) =0.058°.

$$M = \frac{5.12 \times 4.69}{0.058} = 414,$$

$(C_5H_8)_6$ requires 408.

Treatment with Thionylchloride

To one gram of the substance was added 13 c.c. of thionylchloride and heated at 240° for 30 minutes. The product, after the mother liquor was driven off by suction, was washed with water and recrystallised several times from benzene, melting at 282–283°. In this treatment the original matter was not changed into an expected product.

Treatment with Phosphorus Pentachloride

To one gram of the substance 2 grams of phosphorus pentachloride were added, fused, and treated with a large amount of water. The resulting product was recrystallised several times from alcohol, and melted with decomposition at 238°, getting dark brown at 230°. The analytical results of this substance were as follows:

Subst. 4,399 mg., CO_2 11.060 mg., H_2O 3.620 mg.;

„ 4.585 „ , „ 11.520 „ , „ 3.750 „ ;

C 68.57, 68.52; H 9.21, 9.15%.

Subst. 3.218 mg., Cl 0.623 mg.;

„ 2.152 „ , „ 0.414 „ ;

Cl 19.36, 19.24%

$C_{32}H_{51}Cl_3O$ requires C 68.84; H 9.22; Cl 19.07%.

In this case a substance, which melted at 203° into a light brown-coloured jelly and turned to a brown liquid at 210° was also obtained as a by-product. It was analysed with following results.

Subst. 4.191 mg, CO_2 11.840 mg, H_2O 3.910 mg;

C 77.05; H 10.43%.

The chlorination of miliacin was next tried by adding phosphorus pentachloride into the saturated benzene solution. The resulting product could be separated by fractional crystallisation from alcohol into

two parts, one of them being easily soluble in alcohol, melting at 175–176° into a white milky state and at 183° into a clear liquid and showing needle shaped crystals the other being with difficulty soluble in alcohol, melting at 220–222° into clear liquid, and showing monoclinic hexagonal crystals. The analytical results of the latter substance were as follows:

Subst. 4.939 mg., CO_2 14.245 mg., H_2O 4.690 mg.;
" 3.072 " , Cl 2.226 " ;
Found C 78.66; H 10.63; Cl 7.36%
$C_{32}H_{53}ClO$ requires C 78.55; H 10.93; Cl 7.25%.

The substance melting at 183°, contains about 3 per cent. of chlorine. which shows imperfect chlorination.

Determination of Methoxyl Group

According to the micro-ZEISEL method, the methoxyl group of miliacin was examined with negative result, rendering it evident that the methoxyl group was not present in the miliacin molecule at all.

Zinc Dust Distillation

A glass tube was filled as follows: A plug of glass wool was placed at the constricted end of the tube. This was followed by a 10 cm. layer of pumice about the size of a pea which had been impregnated with zinc dust. The coating of the pumice with zinc dust was accomplished as follows. A paste was prepared by rubbing up 100 grams of zinc dust with 30 c.c. of water. The pumice was added to this and the mixture stirred until the pumice was well covered with the zinc dust paste. The pumice was then removed from the paste and dried.

After the pumice-zinc dust layer was placed a mixture consisting of 4 grams of miliacin and 20 grams of zinc dust, to which some pumice impregnated with zinc dust had been added. Finally a 5 cm. layer of pumice-zinc dust was added.

The distillation experiment was begun by passing through the system a fairly rapid stream of dry hydrogen until all of the air was replaced. The current of the gas was then diminished so that only about three bubbles per second passed through. Heat was then applied nnder the front and rear ends of the tube containing the layers of pumice-zinc dust. Finally the mixture of miliacin and zinc dust was gradually heated until the temperature of the air was at 360°. Throngh-out the experiment the temperature was not permitted to exceed 360°.

Examination of the distillate:—The distillate consisted of a liquid portion together with a resinous substance. From 12 grams of the

original miliacin 5 grams of the resinous substance and 3 grams of a yellowish liquid substance were obtained, which, when distilled under diminished pressure, passed over between 92–98° 5 mm. and had an aromatic odour with the following specific gravity and refractive index:

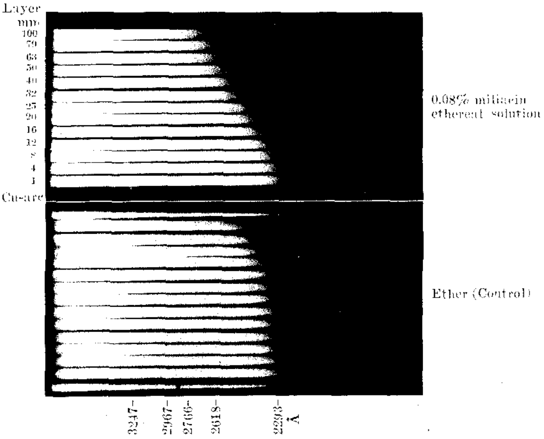
Sp. gr. 0.8988 at 18°; Specific refractivity 1.500 at 25°.

This substance is considered to be a sesquiterpene; further studies on it are in progress.

Examination of the Absorption Spectra

The absorption spectra of miliacin in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as the source of illumination and showed general absorption as indicated in the following figure.

Fig. 2



Properties of Phytosterol

Phytosterol was soluble in ethyl alcohol, anhydrous acetic acid, xylene, benzene, ethyl acetate, ether, chloroform and carbon tetrachloride which melted at 135–136° (not corrected).

Colour reaction:—About 5 miligrams of the sample were dissolved in 2 c.c. of chloroform and 2 c.c. of conc. sulphuric acid added slowly. At that moment the layer of chloroform was represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two. But after a short time, the layer of chloroform turned to a reddish colour while the layer of sulphuric acid to a fluorescent green colour. (HAEGAR-SALKOWSKY).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added slowly. The layer of anhydrous acetic acid assumed a green colour, while the layer of sulphuric acid was a yellow colour near the boundary of the two. (LIEBERMANN-BURCHARD).

Specific Rotatory Power

Phytosterol, recrystallised from alcohol and dried at 110°, was dissolved in chloroform and the specific rotatory power was determined

$$[\alpha]_D^{27°} = \frac{-1.96° \times 100}{6.277 \times 1} = -31.2°.$$

Analysis

The product recrystallised from alcohol was dried at 110° under reduced pressure and lost in weight as follows:

Subst. 4.318 mg., loss in weight 0.092 mg., (2.16%).

The elementary analysis of this dried substance was carried out and the following results obtained:

Subst. 4.236 mg., CO_2 13.095 mg., H_2O 4.612 mg.

Found C 84.31; H 12.18%

Calc. for $C_{27}H_{46}O$ „ 83.86; „ 12.00%.

Phytosterol Acetate

To obtain the phytosterol acetate, the phytosterol was dissolved in a small amount of anhydrous acetic acid, which was heated over a free flame for 2 hours. When it was poured into a large amount of water the acetate was separated, which after several recrystallisations from acetone, melted at 128°.

Specific Rotatory Power:—The specific rotatory power of this product was determined with the following results.
(chloroform as a solvent).

$$[\alpha]_D^{27°} = \frac{-2.05° \times 100}{5.375 \times 1} = -38.1°.$$

Analysis:—

Subst. 4.448 mg., CO_2 13.287 mg., H_2O 4.601 mg.
 „ 4.081 „ , „ 12.213 „ , „ 4.240 „ .

Found C 81.48, 81.62; H 11.57, 11.62%

Calc. for $C_{27}H_{45}OCOCH_3$ .. 81.24 ; .. 11.29 %.

Determination of Acetyl Group:—

Subst. 4.085 mg., N/100-NaOH 1.005 c.c.,
 „ 4.061 „ , „ „ 1.004 „ .

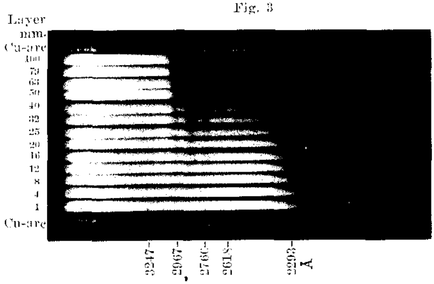
Found $COCH_3$ 11.8, 11.9%

Calc. for $C_{27}H_{45}O \cdot COCH_3$ .. 10.04%.

Determination of the Absorption Bands

The absorption bands of phytosterol in ethereal solution were *estimated with an* ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as the source of illumination, and it was found that this phytosterol contained a small amount of ergosterol. The five absorption bands characteristic to ergosterol are shown in the figure (*i.e. at 293, 282, 270, 260 and 250 μ.μ.*).

Fig. 3



0.386% Phytosterol (in ether).

Separation of Solid and Liquid Fatty Acids

The solid and liquid fatty acids were separated from the previously mentioned saponifiable matters, which had been treated with ether for the removal of unsaponifiable ones by the lead-salt-ether method as follows:

On removing the alcohol as completely as possible, the saponifiable matter was dissolved in a large amount of water, and the solution was

made slightly acidic to litmus paper with acetic acid. To this solution was gradually added with constant shaking a proper amount of a filtered 10 per cent. solution of normal lead acetate, previously heated to boiling point. The flask was put aside to cool; when the lead soaps became hard and cold, the liquid was decanted off through a filter, and any of the lead salts left in the filter returned to the flask. The contents of the flask were washed many times with boiling water. Before pouring off the water, the mixture was carefully cooled each time by a rapid rotation of the flask under a stream of cold water. The flask was finally turned upside down and held over a filter by means of a clamp, till as much of the water as possible had drained off. To this dried lead salt a large amount of ether was added and the flask closed and thoroughly shaken, after which the contents of the flask were allowed to stand overnight, avoiding undue exposure to the air. Then it was centrifuged and repeatedly washed with ether till the layer of the ether showed no colour. By this procedure the lead salts of the liquid fatty acids were separated out from the salts of solid fatty acids. To the solution of the lead salt which had been brought to the separating funnel, was added an excess of hydrochloric acid (1:3). The separating funnel was then securely stoppered and vigorously shaken until lead chloride ceased to settle out. The solid lead salts insoluble in ether were transferred to the second separating funnel by means of a jet of ether. The mixtures in the funnel were treated with an excess of hydrochloric acid (1:3), decomposition of the lead salts being helped by vigorous shaking as in the case of liquid fatty acids.

On running off the aqueous solution, the ethereal solution was washed successively with small quantities of water until the washing water was free from hydrochloric acid, and dried over anhydrous sodium sulphate. The ether of the solution was then distilled off, and residual fatty acids were retained in a vacuum desiccator for further investigation.

From 3.9 kilograms of the natural oil 2.6 kilograms of liquid fatty acids and 0.3 kilograms of solid fatty acids were obtained.

Liquid Fatty Acids

To isolate the individual fatty acid from the liquid fatty acids which had already been obtained, the following experiments were carried out.

Liquid fatty acids were esterified with methyl alcohol, and the methyl esters were then subjected to fractional distillation under dimi-

nished pressure. Fractional distillation was repeated in each fraction and thus successive fractional distillations were made five times. In each fraction the iodine value, refractive index and saponification value of esters were determined, but satisfactory results could not be obtained.

Next, liquid fatty acids were subjected to distillation under diminished pressure, which passed over almost completely between 186–201°/2 mm. Using this distillate, the following bromination and oxydation experiments were carried out.

a) Bromination:—Twenty grams of the liquid fatty acids were dissolved in 200 c.c. of glacial acetic acid and to this bromine added so long as the colour of the bromine was not extinguished. During this procedure it was cooled with ice water. After standing overnight in the ice-chest, the bromides thus produced were introduced into a large amount of water and the resulting bromides were washed with water several times until no more bromine reaction was found. Then they were refluxed with hot petroleum ether, the portion soluble in petroleum ether being thus obtained. Next, the bromides insoluble in petroleum ether were treated with ether and it was found that all of them were dissolved in it. It was thus found that the di- and tetra- bromides were present, but hexa- or higher bromides were not present in them. The following experiments were then made on the bromides which were soluble in petroleum ether and in ethyl ether separately.

1) The bromides soluble in petroleum ether:—

The bromide was dissolved by a small amount of petroleum ether and placed overnight in the ice-chest. Thus the white crystals were separated on the bottom of the vessel and filtered off. This procedure was repeated several times until no more white crystals were separated out. The yellowish solution of petroleum ether was washed with dilute sodium thiosulphate solution to remove the free bromine. Then, to remove the moisture, it was treated with anhydrous sodium sulphate. After distilling off the petroleum ether, a yellowish brown liquid bromide was obtained which was analysed with the following results.

Bromine contents (B. C. method),

Subst. 0.5052 g., AgBr 0.4685 g.

" 0.4277 " , " 0.3981 " ,

Found 39.46, 39.61% of bromine;

Calc. for $C_{18}H_{34}O_2Br_2$ 39.15 " " "

Neutralisation value.

Found 121.4;

Calc. for $C_{18}H_{34}O_2Br_2$ 127.5.

From the above results, the yellowish brown liquid bromide should not be considered to be dibromostearic acid; from the neutralisation value and bromine contents, it seems to be dibromostearic acid mingled with another which has more bromine contents than itself.

2) The bromide insoluble in petroleum ether but soluble in ether:—

The portions of the white crystalline bromide, insoluble in petroleum ether but soluble in ether, were recrystallised from ether several times melting at 113.5–114.5° as is given in the literature. It was analysed with the following results.

Bromine contents (B. C. method):

Subst. 0.2127 g., 0.0992 g.; AgBr 0.2691 g., 0.1236 g.;

	Neutralisation value	Bromine %
Found	94.3	53.84, 53.04
Tetrabromostearic acid	93.5	53.27

From the above, the solid white crystalline bromide, insoluble in petroleum ether but soluble in ether, is tetrabromostearic acid.

b) Oxidation:—A certain amount of liquid fatty acids was taken and neutralised with 3 per cent. sodium hydroxide solution and introduced into two litres of water stirring vigorously to dissolve them. It was then slightly acidified with dilute acetic acid, cooled to 0°, and 2 litres of 1.5 per cent. potassium permanganate solution, previously cooled to 0°, was added from the burette with continuous stirring. Then it was kept overnight in the ice-chest. To remove the free potassium permanganate a saturated sodium sulphide solution was added to this. The resultant white mass was filtered, washed with water several times and dried over a $CaCl_2$ under diminished pressure. To remove the unoxidised oil, the dried mass was treated with petroleum ether, using Soxhlet's apparatus. The residue was refluxed with hot ether. The substance soluble in ether was recrystallised several times from alcohol, melting at 131–132°, and analysed with the following results.

Subst. 0.1101 g., CO_2 0.2758 g., H_2O 0.1143 g.;

	Neutralisation value	Molecular weight	C %	H %
Found	177.5	316.0	68.32	11.62
Calc. for $C_{18}H_{34}(OH)_2O_2$	177.4	316.3	68.29	11.47

The product thus obtained, as is seen from the above table, coincides

with dihydroxystearic acid. This fact shows that oleic acid is present in the liquid fatty acids.

The oxidised substance insoluble in ether was repeatedly recrystallised from alcohol and a substance obtained which melted at 173–174°, being identified as sativic acid. The substance was analysed.

Subst. 0.1035 g., CO_2 0.2358 g., H_2O 0.0990 g.;

	Neutralisation value	C %	H %
Found	159.7	62.13	10.70
Calc. for $C_{18}H_{32}(OH)_4O_2$	161.1	62.02	10.42

Considering the above bromination and oxidation, the following conclusions may be made.

1) Linolenic acid or higher fatty acids are not present in the liquid fatty acids.

2) The liquid fatty acids are composed largely of linolic, iso-linolic and oleic acids.

Solid Fatty Acids

To isolate the individual fatty acid from the mixed solid ones, the following procedure was carried out.

At first a fractional precipitation of fatty acids in alcohol was carried out by adding a small amount of water at a time, but satisfactory results were not obtained. Adding magnesium acetate to the alcoholic solution, fracional precipitation of the fatty acids was tried, but it was not fruitful. The solid fatty acids were then subjected to fractional distillation under 2 mm. pressure. The neutralisation values of the each distillate were measured and those distillates having nearly equal neutralisation values were united and further subjected to distillation. This treatment was carried out eight times successively. Each distillate was recrystalliesd several times from 70 per cent. alcohol; palmitic and carnaubic acids were isolated.

1) The one, which had a neutralisation value of about 220 after 8 redistillations, were recrystallised from 70 per cent. alcohol in lustrous scale like crystals melting at 62–62.5°. When it was mixed with an authentic specimen of palmitic acid, no change in the melting point was observed. Its neutralisation value was 218.9 and its analytical results were as follows:

Subst. 0.1051 g., CO_2 0.2893 g., H_2O 0.1195 g.;

Found C 75.07; H 12.72%;

Calc. for $C_{16}H_{32}O_2$ „ 75.00; „ 12.50 „.

Then it was esterified with methyl alcohol, and the resulting ester melted at 29.5–30.0°. It was found that the main fraction of solid fatty acids having the neutralisation value about 220, consisted of palmitic acid which must have been present in a considerably larger proportion.

2) On fractional distillation, the acids distilled over 200° under 2 mm. pressure, were collected and a further 8 times subjected to fractional distillation. Then it was recrystallised from hot 90 per cent. alcohol and the purest substance obtained, which melted at 73.5–74.5°. This acid was sparingly soluble in cold methyl alcohol, but easily soluble in ether, benzene, acetone, gracial acetic acid and hot alcohol. The substance was analysed.

Subst. 4.547 mg., CO_2 13.030 mg., H_2O 5.340 mg.;
" 4.970 " , " 14.230 " , " 5.810 " ;
Found C 78.15, 78.09; H 13.14, 13.08%;
Calc. for $C_{24}H_{48}O_2$ " 78.18 ; " 13.13%.

From the melting point, solubility and analysis, this acid was considered to be carnaubic acid. According to STÜRCKE[18], the melting point of this acid was 72.5°, according to MAYER and ECKERT[14], 74°, and to DUNBAR and BINNEWIES[8], 72.2°. It was treated with lead acetate and lead salt obtained after several recrystallisations from toluen, sintering at 111° and melting at 114–116°.

To obtain methyl ester from this acid, 0.1 gram of the acid was introduced into an excess of thionylchloride, which was heated at 100–120° in an oil-bath for about an hour. After the excess of thionylchloride had been driven off under reduced pressure, the chloride thus produced was introduced into methyl alcohol, which was recrystallised several times from ethyl acetate, sintering at 54° and melting at 56–57°. The tribromanilide of carnaubic acid was prepared according to the general method of ROBERTSON[17] as follows:—0.1 gram of the substance was added to an excess of thionylchloride which was heated between 100–120° on the oil-bath using a reflux condenser for about an hour. This was then treated with a little more tribromaniline than calculated, and was heated for about half an hour at 120–130°. The resulting product was recrystallised several times from alcohol and further from petroleum ether which melted at 115.5–116.5° (not corrected).

The bromine contents of this product were estimated:—

Subst. 4.501 mg., AgBr 3.770 mg.
Found 35.64% of bromine.
$C_{23}H_{47}CO \cdot NHC_6H_2Br_3$ requires 35.25 " " " .

Solid fatty acids, from the above described results, appear to consist chiefly of palmitic acid and carnaubic acid but the former greatly predominating.

Chapter II

On the Oil of *Syntherisma sanguinalis* Dulac var. *ciliaris* Honda (*'mehishiba'*)

The seeds of *'mehishiba'* were collected near Kasamatsu-chō in Gifu Prefecture in September, 1931. This plant grows in this country in the wilderness. The seeds were dried in sunlight, ground by means of a stone mill and the oil extracted from them with ether by continuous percolation. From 9 kilogram (moisture 12.56%) of the ground material 330 grams of the oil were obtained, which were 3.7 per cent. of the original material.

The general analysis of the ground material were as follows:

Moisture	12.56%
Ash	7.10 ,,
Crude fat	3.70 ,,
Crude protein ($N_2 \times 6.25$)	12.03 ,,
Total carbohydrate (glucose $\times 0.9$)	48.50 ,,

The unsaponifiable matters consisting largely of sterols and miliacin were estimated and solid and liquid fatty acids in the oil also determined by the following method: 14.7 grams of the oil were hoiled with alcoholic potassium hydroxide for two hours, diluted with water and extracted five times with ether. The ether was distilled, and the residue, after it had been dried, weighed 2.47 grams, which cotained a considerable amount of miliacin in addition to sterols. Miliacin was obtained after treating the crude unsaponifiable matters with 90 per cent. hot alcohol, which weighed 0.39 gram. Through the lead soap 2.34 grams of saturated and 8.46 grams of unsaturated fatty acids were determined.

The percentages of each substance were as follows:

Unsaponifiable matters	16.75%
Miliacin	2.62 ,,
Solid fatty acids	15.92 ,,
Liquid fatty acids	57.50 ,,

Some constants of the natural oil, solid and liquid fatty acids were determined with the following results.

a) The natural oil.

	...ractive index at 30°	1.4705
	Acid value	104.5
	Saponification value	178.8
	Reichert-Meissl value	4.3
	Polenske value	1.1
	Iodine value	124.7
b)	Solid fatty acids.	
	Melting point	50–52.5°
	Neutralisation value	207.4
	Iodine value	11.3
c)	Liquid fatty acids.	
	Specific gravity 27°/27°	0.9024
	Refractive index at 30°	1.4655
	Neutralisation value	182.6
	Iodine value	134.4

Preparation of Miliacin and Phytosterol

The oil, 300 grams, was saponified by heating on the water-bath with alcoholic potassium hydroxide. The soap solution was diluted with water and extracted with ether. After the distillation of ether, the residue was again boiled with alcoholic potassium hydroxide for two hours. On diluting the solution with water it was extracted with ether and the ethereal solution was washed, dried and filtered. On distilling off the solvent, a dark brown, crystalline residue was obtained which was treated with hot alcohol. Crude miliacin remained in a crystalline state. After several recrystallisations from benzene, 2 grams of miliacin were obtained in colourless hexagonal plates.

The unsaponifiable matters soluble in alcohol were treated with norite, recrystallised from alcohol several times and snow-white crystals obtained which melted at 135–136° and gave a marked LIEBERMANN-BURCHARD reaction. This crystalline substance was not enough in quantity for further investigations.

Miliacin thus obtained from 'mehishiba' melted at 282–283° (not corrected), and when mixed with the miliacin from millet oil no change in melting point was observed. Furthermore, the solubility, the rotatory power, and the colour-reactions were exactly the same as those of miliacin from millet oil.

$$[\alpha]_D^{16°} = \frac{+5.2° \times 100}{2.575 \times 1} = +20.1° \text{ (in chloroform)}$$

Miliacin from millet oil $[\alpha]_D^{25°} = +20.2°$

Analysis

1. Subst. 4.235 mg., CO_2 13.025 mg., H_2O 4.570 mg.
2. „ 4.180 „ , „ 12.881 „ , „ 4.524 „ .

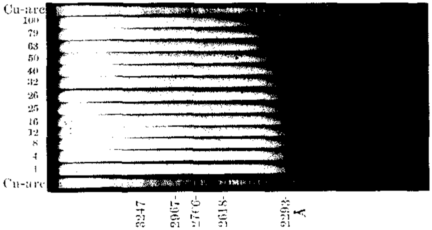
Found C 83.88, 84.04; H 12.08, 12.11%

$C_{32}H_{54}O$ requires „ 84.50 ; „ 11.98%.

Determination of the Absorption Bands

The absorption bands of miliacin in ethereal solution were estimated with an ADAM HILGAR E type quartz spectrograph, using a hydrogen GEISSLER tube as a source of illumination and showed general absorption as indicated in the following figure.

Fig. 4



miliacin from 'mehishiba' 0.08% ethereal solution.

The Liquid and Solid Fatty Acids

The isolation and the identification of the solid and liquid fatty acids were made by the same treatments as detailed in the case of millet. From 300 grams of natural oil 160 grams of liquid fatty acids and 45 *grams of solid fatty acids were obtained.*

On bromination of the liquid fatty acids di- and tetra- bromostearic acid (m.p. 114–115°) were produced, which shows the presence of oleic and linolic acids in the oil of '*mehishiba*'.

Analytical data.

Dibromostearic acid.

Bromine content (B. C. method).

Subst. 0.3244 g., AgBr 0.3013 g., Bromine 39.53%*

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* It seems that the dibromostearic acid was contaminated with some compound whose bromine contents are greater than its own.

Calc. for $C_{18}H_{34}Br_2O_2$ 36.16 „

Tetrabromostearic acid.

Bromine contents (B. C. method).

Subst. 0.2658 g., AgBr 0.3323 g., *Bromine* 53.21%

Calc. for $C_{18}H_{32}Br_4O_2$ 53.28 „

From the solid fatty acids palmitic and carnaubic acids were isolated *as in the case of millet oil.*

Analytical data.

Palmitic acid.

Subst. 4.669 mg., CO_2 12.715 mg., H_2O 5.314 mg.

Found C 74.27; H 12.74%.

Calc. for $C_{16}H_{32}O_2$ „ 75.00; „ 12.50 „.

Carnaubic acid.

Subst. 4.171 mg., CO_2 12.018 mg., H_2O 4.972 mg.

Found C 78.58; H 13.34%.

Calc. for $C_{24}H_{48}O_2$ „ 78.18; „ 13.13 „.

CHAPTER III

On the Oil of *Echinochloa crusgalli* BEAUV. subsp. *edulis* HONDA var. *typica* HONDA (*Japanese Barnyard Millet*)

The material employed in this investigation was produced in Gifu Prefecture during the preceding year, and was ground by means of a *stone mill, the oil being extracted by continuous percolation with hot* benzine. From 48.8 kilograms (moisture 14.7%) of the ground material 2.5 kilograms of the oil were obtained, which constituted 5.1 per cent. of *the original materials.*

Some constants of the natural oil were determined and the following results obtained.

Specific gravity (15°)	0.914
Refractive index (30°)	1.467
Acid value	121.25
Saponification value	202.32
Iodine value	117.79
Hehner value	92.80
Reichert-Meissl value	0.52
Polenske value	1.4
Acetyl value	62.11

The products insoluble in acetone, unsaponifiable matters, solid and liquid *fatty acids in the oil were determined by the following*

method:—The oil was treated by twenty times its weight of acetone and a precipitate was produced, which was collected, dried in a vacuum over sulphuric acid, and weighed. From the portion soluble in acetone, the content of unsaponifiable matters were determined by BÖMER's method. The contents of the solid and liquid fatty acids in the residual portion from which had been removed the unsaponifiable matter were determined by the lead-soap-ether method. They were analysed with the following results:

Subst. g.		Precipitate by acetone g.	Unsaponifiable matter g.	Solid fatty acid g.	Liquid fatty acid g.
I.	18.08	0.3805 (2.10%)	1.062 (5.87%)	1.143 (6.32%)	13.96 (77.21%)
II.	16.79	0.3782 (2.26%)	0.930 (5.54%)	1.158 (6.89%)	12.94 (77.07%)

Some properties of the solid and liquid fatty acids thus obtained were as follows:—

Fatty acids		Refractive index at 30°	Iodine value	Neutralisation value
Solid	I.	—	14.47	213.48
	II.	—	15.06	213.42
Liquid	I.	1.4630	137.02	196.15
	II.	1.4630	138.48	195.62

Separation of Crusgallin, Hiyeol, and Phytosterol

One hundred gram batches of the oil were introduced into about 1,000 grams of acetone, stirred for a while and left stand in the ice-chest for about 3 hours. The resultant brownish precipitate was collected and dried in a vacuum desiccator. This product was extracted with a SOXHLET's apparatus, using petroleum ether (b. p. 40–60°) for more than one week. From 2.1 kilograms of oil, 4 grams of crude crusgallin were obtained. After the removal of the solvent from the portion soluble in acetone, the residue, from which the unsaponifiable matters were to be isolated, BÖMER's method was applied. On removing the ether from the extract which contained the unsaponifiable matters, the residual alcohol solution was kept in a dark room for about a week, when a great deal of the yellow mass was separated and dried in a vacuum desiccator. This material was treated with 95 per cent. alcohol and roughly divided into three portions, that easily soluble (I), that with difficulty soluble (II), and that insoluble (III) in alcohol.

After treating the portion (I) with norite and obtaining several recrystallisations from 95 per cent. alcohol, a small amount (about 0.5 g.) of a product was obtained, which crystallised in needles, melting at 91.5°.

This product was named "hiyeol" and is regarded as a new compound.

The portion (II) was repeatedly crystallised from alcohol and a *phytosterol was obtained in thin hexagonal plates, melting at 136.5–137.5°* (not corrected). The total yield of this product was about 5 grams. From this portion another product which was comparatively *less soluble in alcohol than phytosterol was isolated. After several re*crystallisations from alcohol, this product melted at 121–124°, weighed 2.4 grams, but was not pure enough as the melting point indicates.

From the portion (III) insoluble in alcohol, and also from the above-mentioned crude crusgallin, after several recrystallisations from benzene, pure crusgallin was obtained which weighed 0.8 gram, melting *at 280° (not corrected).*

From this portion (III), also another product, through several recrystallisations from chloroform, was obtained, which weighed 1 gram, *melting at 62–63°. This product may not be a pure substance, for it* showed white turbidity at its melting point.

Properties of Crusgallin

Crusgallin crystallises in colourless hexagonal plates belonging to

Fig. 5

Crusgallin

the monoclinic sytsem as mentioned below, and melting at 280° *(not*

corrected). It is considered likely that crusgallin is different from miliacin, for when mixed with miliacin, a change in the melting point (about at 250°) was observed.

Crusgallin is soluble in ether, chloroform, carbontetrachloride, xylene, benzene, scarcely soluble in methyl and ethyl alcohol, anhydrous acetic acid, ethyl acetate, acetone; and insoluble in cold alcohol or water.

Colour reaction:—About 5 mg. of the substance were dissolved in 2 c.c. of chloroform, and conc. sulphuric acid added slowly to the solution; immediately the layer of chloroform was represented by a white turbidity, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. But after a while the layer of chloroform turned reddish, while the layer of sulphuric acid, a fluorescent green colour. (HAEGAR-SALKOWSKY's reaction).

A small amount of the substance was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added slowly. The layer of anhydrous acetic acid assumed a violet red which in a short time turned to a wine-red, while the layer of sulphuric acid showed no colour. (LIEBERMANN-BURCHARD's reaction).

Specific Rotatory Power

The specific rotatory power of the substance was determined with the following results. (chloroform as a solvent).

$$[\alpha]_D^{26} = \frac{+0.44° \times 100}{1.350 \times 2} = +16.3°.$$

Analysis:—

Subst.	4.759 mg.,	CO_2	14.710 mg.,	H_2O	4.950 mg.;	
,,	5.002 ,, ,	,,	15.490 ,, ,	,,	5.290 ,, ;	
Found		C	84.30, 84.46;	H	11.64, 11.83%;	
$C_{32}H_{54}O$ requires		,,	84.50;	,,	11.98 ,, ;	
$C_{31}H_{52}O$	,,	,,	84.47;	,,	11.90 ,, ;	
$C_{30}H_{50}O$	,,	,,	84.43;	,,	11.82 ,, ;	
$C_{29}H_{48}O$	,,	,,	84.39;	,,	11.73 ,, ;	
$C_{28}H_{46}O$	,,	,,	84.37;	,,	11.64 ,, ;	

Molecular weight

The determination of the molecular weight was carried out according to RAST's camphor method and the cryoscopic method, with the following results:

1) RAST's method;

Subst. 0.015 g.; camphor 0.15 g.; $\Delta t = 9°$;

$$Molecular\ weight = \frac{40 \times 100}{9} = 444.$$

$C_{32}H_{54}O$ requires .... 454.

2) Cryoscopic method;

Subst. 0.04 g.; benzene 21.25 g.

Depression of freezing point $(\Delta t) = 0.020°$

$$\text{Molecular weight} = \frac{5.12 \times 1.88}{0.020} = 481.$$

$C_{32}H_{54}O$ requires .... 454.

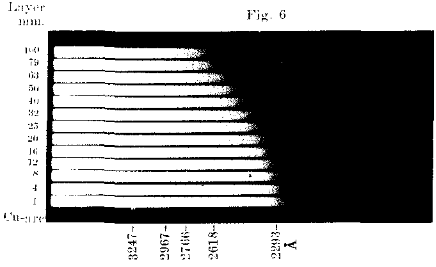
Catalytic Hydrogenation

Catalytic hydrogenation was tried for about 24 hours using a mixture of 0.1 grom of the substance, 15 c.c. of hexane and 0.1 gram of platinum black, only to discover no absorption of hydrogen. The recovered substance, after recrystallisation from benzene, melted at 280° (not corrected): when mixed with the original substance no depression of the melting point ensued.

Examination of the Absorption Spectra

The absorption spectra of crusgallin in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph using a hydrogen GEISSLER tube as the source of illumination. General absorption was found as indicated in the following figure.

Fig. 6



Crusgallin 0.08% ethereal solution

Crystallographic Studies

The author desires to express his hearty thanks to Mr. TAKEHIKO SHIGA, for his kindness in making the crystallographical observations.

Fifteen well developed crystals had been selected, from which eight crystals having clear reflecting images of the slit were chosen and V. GOLDSCHMIDT's two-circle goniometer was used. Then taking the largest crystal-face as the pole, ρ' and φ' of each face were measured, and the faces were projected by stereographic projection, from which the symmetry was examined. It was found that this crystal belongs to the monoclinic system and has perhaps a holohedral symmetry. Next, from *the facts that the symmetry-plane of a monoclinic crystal is (010)*, the best-developed face was taken as the hase and the next well-developed face as (100). From this assumption the numerical value of the angle β was found to be 99°13′. The values of ρ and φ were recalculated from the measured values of ρ' and φ' so as to take (001) zone as the fundamental circle of projection, and the faces were projected. The results are shown in the figure 7 and 8 and the following table.

The axial ratio was calculated, taking a pyramidal face of this crystal as the unit face e. g. ($\bar{1}$11) with the following results: a:b:c= 1.5110:1:1.49596. Angles which were measured and calculated were as follows:

Crystal face	Calculated values		Observed values		n
	ρ	φ	ρ	φ	
001	*		9°13′	—	15
100	90°00′	90°00′	90°00′	90°00′	16
302	79° 1′	90°00′	79°00′	90°00′	5
111	*	*	79°13′	23°51′	17

The mark (*) shows the angles which had been used to calculate the axial ratios and n the uumbers of the measured faces. The forms which appeared in all crystals were (001), (100) and (111), and the form (302) was rare.

A crusgallin crystal possesses two cleavages, one parallel to (001), more perfect, and the other parallel to (100).

The cleavage-flake of this substance is flexible, and the reflection of light by the crystal face was strongest on (001) and weakest on (302).

The horizontal stria on the face (100) are the traces of the cleavages parallel to (001).

Figure 8 shows an ideal illustration of the average habit and type of development of the crystal faces.

Fig. 7
Stereographic Projection of Crusgallin

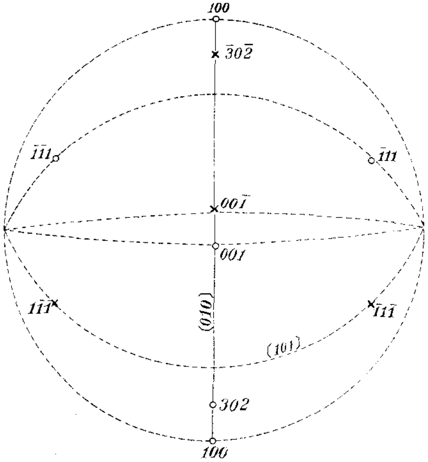
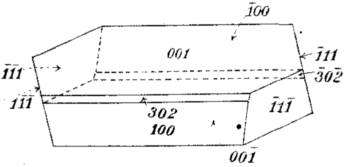


Fig. 8
Crystal Form of Crusgallin



Properties of Phytosterol

The substance is soluble in methyl and ethyl alcohol, anhydrous acetic acid, xylene, benzene, ethyl acetate, acetone, ether, chloroform and carbontetrachloride, and melts at 136.5–137.5° (not corrected). When it was mixed with a phytosterol from millet (*Panicum miliaceum* L.) the melting point was 136°.

Colour reaction:—About 5 miligrams of the substance were dissolved in 2 c.c. of chloroform to which 2 c.c. of conc. sulphuric acid were added gently. On that instance the layer of the chloroform was represented by a white turbidity, while the layer of sulphuric acid was yellow colour near the boundary of the two layers. But after a short time the layer of chloroform was changed into a reddish colour, while the layer of sulphuric acid changed to a fluorescent green colour. (HAEGAR-SALKOWSKY reaction).

A small amount of the sample was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added gently. The layer of anhydrous acetic acid assumed a green colour, while the layer of sulphuric acid was a yellow colour near the boundary of the two layers. (LIEBERMANN-BURCHARD reaction).

Specific Rotatory Power

The compound, melting at 136.5–137.5°, was dried at 110° and dissolved in chloroform. The specific rotatory power of this solution was determined with the following results.

$$[\alpha]_D^{25^\circ} = \frac{-1.07^\circ \times 100}{4.862 \times 1} = -22.0^\circ$$

Analysis

The substance recrystallised from alcohol was dried at 110° under reduced pressure and lost in weight about 3 per cent.

Subst. 4.475 mg., Loss in weight 0.137 mg. (3.06%).

The anhydrous substance was analysed.

Subst.	0.1004 g.,	CO_2 0.3074 g.,	H_2O 0.1078 g.
,,	0.1062 ,, ,	,, 0.3252 ,, ,	,, 0.1152 ,,
,,	4.338 mg.,	,, 13.368 mg.,	,, 4.725 mg.

Found C 83.50, 83.51, 84.04; H 12.01, 12.14, 12.19%.

Calc. for $C_{27}H_{46}O$,, 83.86; ,, 12.00 ,, .

Molecular Weight

The determination of the molecular weight of this product was carried out according to RAST's camphor method with the following results:

Subst. 8.2 mg., Camphor 84.6 mg., $\Delta t = 10^\circ$;

$$\text{Molecular weight} = \frac{40 \times 970}{10} = 388.$$

Calc. for $C_{27}H_{46}O$ 386.

Phytosterol Acetate

The phytosterol dissolving in a small amount of anhydrous acetic acid was heated over a free flame for 2 hours. On pouring it into a large amount of water, the acetate was separated and recrystallised from *ethyl acetate, melting at 124–126° (not corrected).*

Specific Rotatory Power:—The specific rotatory power of this product was determined in chloroform.

$$[\alpha]_D^{25°} = \frac{-1.44° \times 100}{5.550 \times 1} = -26.0°$$

Analysis:—

Subst. 4.173 mg.,	CO_2 12.513 mg.,	H_2O 4.405 mg.;	
„ 4.263 „ ,	„ 12.791 „ ,	„ 4.444 „ ;	
Found		C 81.78, 81.83;	H 11.81, 11.67%;
Calc. for $C_{27}H_{45}O \cdot COCH_3$ „		81.24;	„ 11.29%.

Determination of Acetyl Group:—This determination was carried out after the method of PREGL and SOLTYS.

Subst. 4.002 mg., $N/100$	1.118 NaOH	0.97 c.c.
„ 3.892 „ ,	„	0.94 „ ;
Found	CH_3CO	11.7, 11.6%,
Calc. for $C_{27}H_{45}O \cdot COCH_3$	„	10.04%.

Examination of the Absorption Spectra

The absorption spectra of the phytosterol in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph, using a *hydrogen GEISSLER tube as the source of illumination*; it was found that

Fig. 9

'*Hiye*'-Phytosterol 0.386% ethereal solution

this phytosterol contains a small amount of ergosterol for the five absorption bands shown in the figure are characteristic of ergosterol (i.e. at 293, 282, 270, 260 and 250 $\mu\mu$).

Properties of Hiyeol

Hiyeol is very readily soluble in methyl and ethyl alcohol, acetic acid, anhydrous acetic acid, ethyl acetate, acetone, ether, chloroform, benzene, xylene and carbon tetrachloride, and crystallised out from alcohol in needles, melting at 91.5° (not corrected).

Colour reaction:—A small amount of the substance was dissolved in anhydrous acetic acid, to which conc. sulphuric acid was added with caution. The layer of anhydrous acetic acid assumed a deep red colour.

Specific Rotatory Power

Hiyeol was dissolved in a chloroform and a determination of its specific rotatory power gave the following result:

$$[\alpha]_D^{27°} = \frac{+1.9 \times 100}{3.08 \times 1} = +61.9°.$$

Analysis

The product, recrystallised from alcohol, was analysed and the following results were obtained.

Subst. 4.802 mg., CO_2 14.860 mg., H_2O 5.030 mg.

Subst.	4.802 mg.,	CO_2 14.860 mg.,	H_2O 5.030 mg.	
,,	5.090 ,, ,	,, 15.740 ,, ,	,, 5.420 ,, .	
Found		C 84.40, 84.34;	H 11.72, 11.92%;	
$C_{32}H_{54}O$ requires	,,	84.50;	,,	11.98 ,, ;
$C_{31}H_{52}O$,,	,,	84.47;	,,	11.90 ,, ;
$C_{30}H_{50}O$,,	,,	84.43;	,,	11.82 ,, ;
$C_{29}H_{48}O$,,	,,	84.39;	,,	11.73 ,, ;
$C_{28}H_{46}O$,,	,,	84.37;	,,	11.64 ,, .

Molecular Weight

The molecular weight of hiyeol was determined according to RAST's camphor method and the cryoscopic method with the following results:

1) RAST's method:—

Subst. 0.016 g., camphor 0.15 g., $\Delta t = 10°$;

$$M. = \frac{40 \times 106}{10} = 424.$$

2) Cryoscopic method :—

(i) Subst. 0.1036 g., benzene 21.57 g., $\Delta t = 0.056°$;

$$M. = \frac{5.12 \times 4.803}{0.056} = 439.$$

(ii) Subst. 0.1728 g., benzene 14.92 g., $\Delta t = 0.140°$;

$$M. = \frac{5.12 \times 11.58}{0.140} = 423.$$

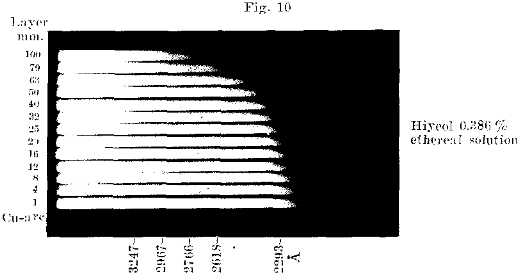
		Molecular weight
$C_{32}H_{54}O$	requires	454
$C_{31}H_{52}O$	,,	440
$C_{30}H_{50}O$	,,	426
$C_{29}H_{48}O$	,,	412
$C_{28}H_{46}O$	,,	398

Examination of Absorption Spectra

The absorption spectra of hiyeol in ethereal solution were examined with an ADAM HILGAR E type quartz spectrograph using a hydrogen GEISSLER tube as the source of illumination and the general absorption shown as indicated in the figure.

Judging from the above described results of the analysis and determination of the molecular weight, the differences of numerical value between hiyeol and crusgallin being within the experimental error, these two substances might be isomeride.

Fig. 10



The value of hiyeol, however, was also close to the one of $C_{30}H_{50}O$ or of $C_{29}H_{48}O$.

Separation of Solid and Liquid Fatty Acids

The solid and liquid fatty acids obtained from the oil by saponification were separated by the lead-salt-ether method. The isolation procedure was carried out with the same treatment as detailed in the case of millet (*Panicum miliaceum* L.).

From 2.2 kilograms of the natural oil 1.5 kilograms of liquid fatty acids and 0.16 gram of solid fatty acids were obtained.

Liquid Fatty Acids

To isolate the individual fatty acid from the liquid fatty acids previously obtained, the following experiments were carried out.

The liquid fatty acids were esterified with methyl alcohol, and the methyl esters were then subjected to fractional distillation under diminished pressure. Each fraction was fractionated and thus successive fractional distillations were made five times. The iodine value, refractive index and saponification value of the esters were determined, but satisfactory results could not be obtained. The liquid fatty acids were then distilled under diminished pressure, and passed over almost completely between 186–210°/2 mm. Using this distillate the following bromination and oxidation experiments were made.

a) Bromination:—The bromination procedure of the liquid fatty acids was carried out in the same way as in the case of millet (*Panicum miliaceum* L.).

The bromide thus obtained was treated with petroleum ether to divide it into two portions—soluble and insoluble. The bromides that were insoluble in petroleum ether were then treated with ethyl ether. It was found that all of them were dissolved in it. This furnished evidence that di- and tetra- bromides were present while hexa- or higher bromides were not present in them. The following investigations were made on the bromides which were soluble in petroleum ether and in ethyl ether separately.

(i) The bromides soluble in petroleum ether:—In order to purify these bromides, they were dissolved in a small amount of petroleum ether and placed overnight in the ice-chest. The white crystals thus produced were filtered off. These treatments were repeated several times until no more white crystals were separated out. The yellowish brown bromide in petroleum ether was washed with dilute sodium thiosulphate solution to remove the free bromine. Then to remove the

moisture, it was treated with anhydrous sodium sulphate. The yellowish brown *liquid bromide* was obtained by removing the petroleum ether, which was analysed with the following results:

Bromine contents (B. C. method),

Subst. 0.4182 g., AgBr 0.3767 g.,

" 0.4862 " , " 0.4413 " ;

	Neutralisation value	Molecular weight	Bromine contents	
Found	120.1	467.2	38.36,	38.63%
Calc. for $C_{18}H_{34}Br_2O_2$	127.5	440.1	36.15%	

From the above results, the yellowish brown liquid bromide is not considered to be dibromostearic acid, but considering its molecular weight and bromine contents, it seems to be a dibromide contaminated with some compound whose bromine contents are greater than its own.

(ii) The bromide insoluble in petroleum ether but soluble in ether:—The product was recrystallised from ether until it melted constantly at 114–115° and analysed with the following results:

Bromine contents (B. C. method),

Subst. 0.3268 g., AgBr 0.4121 g.

" 0.3439 " , " 0.4299 " ;

	Neutralisation value	Molecular weight	Bromine contents	
Found	95.9	585.1	53.67,	53.22%
Calc. for tetrabromostearic acid	93.5	599.9	53.28%	

From the above table, white crystalline bromide which is insoluble in petroleum ether but soluble in ether is shown to be tetrabromostearic acid. The liquid fatty acids therefore contain linolic acid.

b) Oxidation:—The oxidation procedure of liquid fatty acids was performed in the same way as in the case of the one of millet according to Hazura's method. The oxidised white mass thus secured was dried in a $CaCl_2$ desiccator under diminished pressure. To remove unoxidised oil, the white dried mass was treated with petroleum ether, using SOXHLET's apparatus. Then the oxidised fatty acids thus obtained were extracted with ether for 6 days to obtain a compound soluble in ether, which was recrystallised from alcohol, melting at 131–132°, and analysed with the following results:

Subst. 0.1018 g., CO_2 0.2548 g., H_2O 0.1016 g.;

	Neutralisation value	Molecular weight	C%	H%
Found	176.7	317.6	68.26	11.17;

Calc. for dihydroxy-stearic acid	177.4	316.3	68.29	11.47.

The compound thus obtained coincides with dihydroxystearic acid although its melting point is a little lower than that given in the literature. This fact gives evidence of the presence of oleic acid. Next, the oxidised substance insoluble in ether was recrystallised from alcohol and two substances obtained one melting at 156–157° and the other at 172–173°.

Considering the above bromination and oxidation experiments, the following conclusions follow.

(1) Linolenic acid or higher fatty acids are not present in the liquid acid.

(2) The liquid fatty acids are composed largely of linolic, isolinolic and oleic acids.

Solid Fatty Acids

To isolate the individual fatty acid from the mixed solid ones, the following procedure were applied. At first a fractional precipitation from their alcohol solntion was carried out by adding a small amount of water at a time, but satisfactory results were not given. Fractional precipitation of magnesium salts was next made with unsatisfactory results. Then the solid fatty acids were subjected to fractional distillation under 2 mm. pressure. The neutralisation values of each distillate were measured and those distillates which had nearly equal values were nnited and fractionated. This successive treatment was carried out six times. Each distillate was recrystallised from 70 per cent. alcohol, when it was separated in a colourless scale like crystals, melting at 62–62.5°. When mixed with an authentic specimen of palmitic acid, KAHLBAUM "zur Analyse", no change in melting point was observed. Its neutralisation value was 218.9 and the results of the analysis were as follows:

Subst. 0.1022 g., CO_2 0.2814 g., H_2O 0.1410

Found C 75.09; H 12.61%;

Calc. for $C_{16}H_{32}O_2$ „ 75.00; „ 12.50 „.

From the above mentioned facts, the solid fatty acids proved to consist chiefly of palmitic acid.

The author desires to express his great indebtedness to Prof. T. TADOKORO in the Hokkaido Imperial University for his encouragement, to Mr. T. KIKUCHI, the chief engineer of the Hōnen Oil Co., Ltd. for extracting the oils, and to the Department or Education for grants

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STUDIES ON THE DWARF DISEASE OF RICE PLANT

By

Teikichi Fukushi

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I. Introduction

The dwarf disease has long been recognized as one of the most important rice plant diseases in Japan. It is exclusively confined to Japan as far as the writer is aware. About 40 years ago historic severe outbreaks occurred devastating the rice fields in several localities including the Prefectures of Shiga, Kyôto, Hyôgo, Okayama, Hiroshima and Shizuoka. Since then the disease has become more and more widespread throughout the middle and southern part of the Main Island of Japan, Shikoku and Kyûshû and has been responsible for heavy losses in many localities.

Among the earlier investigators of this disease varying opinions were prevalent concerning the nature of this trouble, some workers ascribing it to some unfavourable soil conditions, while others looked upon it as the injury caused by the leaf hoppper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL.

According to our present krowledge this disease is undoubtedly a virus disease transmissible through the agency of the leafhopper above mentioned, acting as the vector. This is the first virus disease of plants shown to be transmitted by an insect as pointed out by KUNKEL (1926)[111] based upon the writer's information, and also by HINO (1927).[76]

During recent years no definite progress has been made with the problem of transmission, host range, and the etiology of the disease. Since this deserves more attention, the writer attempted to attack the problem from the virological point of view.

The present work was begun at the suggestions of Dr. YAMADA, Dean of the Tottori Agricultural College and a part of the experiments

herein recorded, was performed in the Phytopathological Laboratory of that College. The work subsequent to 1929 has been carried out under the general supervision of Prof. S. Ito in the Botanical Institute of the Faculty of Agriculture of the Hokkaido Imperial University. The writer wishes to express here his sincere gratitude to Dr. K. Miyabe, Professor emeritus of the Hokkaido Imperial University and Dr. S. Ito, Professor of plant pathology and also to Dr. S. Matsumura, Professor of entomology of the Hokkaido Imperial University, Dr. G. Yamada, Dean of the Tottori Agricultural College, Dr. Y. Tochinai, Professor of plant pathology and Mr. T. Hayashi, Assistant Professor of Zoology of the Hokkaido Imperial University for their valuable suggestions and kind advices. Also to Mr. S. Mizuno, veteran farmer in the Prefecture of Tottori, Dr. I. Hino, Professor of the Miyazaki College of Agriculture and Forestry, Mr. K. Kuribayashi, Plant Pathologist of the Nagano Agricultural Experiment Station, Mr. M. Sakurai, Plant Pathologist of the Shiga Agricultural Experiment Station and Mr. S. Endo, Assistant Professor of the Miyazaki College of Agriculture and Forestry to whom he is indebted for the materials which they kindly supplied to the writer.

II. History and geographical distribution of the disease

Although the dwarf disease of rice plant must have been existing for a number of years, it had attracted little attention until towards the end of the last century when serious outbreaks of the disease occurred in several localities including the Prefectures of Shiga, Kyôto, Hyôgo, Okayama, Hiroshima, and Shizuoka.

According to Takata (1895–96)[219] the disease was first definitely noted in 1883 in a certain locality in the Prefecture of Shiga. It had spread through the entire Prefecture by 1892, when an area of over 1100 acres of rice fields was invaded by the disease. The destructive nature of the trouble having been realized, the study of the disease was taken up by the Government of Shiga Prefecture in 1893 and intensive work was done in the experimental fields selected in six counties to investigate the disease and to devise control measures. Consequently Takata who took charge of this work and Hashimoto in 1894–95 discovered the causal relation of leafhoppers to the dwarf disease of rice plant. The work was continued by the Shiga Agricultural Experiment Station soon after its establishment in 1895 and by a series of experiments carried out in 1897–1906 it was demonstrated that the disease is transmitted by the leafhopper, *Nephotettix apicalis* Motsch. var.

cincticeps UHL. and that the disease can be effectively controlled by eliminating the leafhoppers in the rice fields. In recent years the loss due to this disease has been remarkably reduced in this Prefecture by the control of the leafhoppers.

In the Prefecture of Kyôto the disease was first noted in 1886 and became prevalent during the following years. There were great outbreaks of the disease in 1889 and 1900 in this Prefecture. During recent years, however, the trouble has been reduced to a minimum by means of the control of the leafhoppers.

In "Rice of the Prefecture of Okayama"[160] issued from the Government of the Prefecture of Okayama in 1910 it is mentioned that the dwarf disease of rice plant seems to have been known for many years in this Prefecture but there is no definite record of the occurrence of the disease until the summer of 1880. During the following years the disease became more widespread and in 1891 an area of over 3200 acres of rice field was invaded by it causing a reduction in yield amounting to 50000 bushels. During recent years, the disease has declined due to the control of the leafhoppers. It was shown hy a survey in 1921 that an area of 70 acres of rice field was threatened by this disease.

In the Prefecture of Hyôgo, there is no authentic record of the occurrence of the disease until 1891, when an area of 7000 acres of rice field was devastated by it.

In the Prefecture of Hiroshima the serious nature of this disease was first realized in 1898–99. It was very severe in 1902–3. In these Prefectures the disease has been effectively controlled in recent years.

The Prefecture of Yamaguchi was invaded by the dwarf disease of rice plant a little later, it being first observed in 1910. Since then the occurrence of the disease has been noted every year though the damage was not very serious.

In the Prefectnre of Shizuoka it is recorded that the disease was very severe in 1892 aud 1893, although its origin is uncertain. During recent years considerable loss has been caused in the rice producing regions along the Tenryû river.

In the Prefecture of Yamanashi the disease was noted in 1888–89 devastating a rice-field area of 350 acres. Later the damage due to this disease was lessened for some unknown reason, but since the great outbreaks in 1915–16 it has again become a menace to the rice crop.

In the Prefecture of Nagano the disease was not noted with certainty until 1892, when an area of 750 acres was infested by it. Since

then the disease seems to have been of general occurrence in this Prefecture and it was shown by a recent survey that an area of 5000 acres of rice field was suffering from this disease.

Beside these, the disease seems to have been known for many years in the Prefectures of Kagoshima, Kumamoto, Nagasaki, Ehime, Saitama, Tochigi and Ibaragi, although its origin is not definitely known.

On the other hand this disease has been first noted during recent years in the Prefectures of Miyazaki, Shimane and Tottori.

In the Prefecture of Miyazaki it was first noted in 1912–13 but was confined to certain localities until 1918. Since then it became more prevalent year after year inducing at length the great outbreak of 1930, which infested an area of 137,500 acres or 95 percent of the rice fields in this Prefecture and caused a total loss of 395,000 bushels.

In the Prefecture of Shimane the disease first appeared in 1914 and in the same season or a year later it was noted to occur in the Prefecture of Tottori.

It is worthy of note that dwarf disease of rice plant has declined during recent years in the Prefectures of Shiga, Kyôto, Hyôgo, Okayama and Hiroshima where the rice crop seriously suffered from the disease for a number of years. The disease has in recent years been destructive in the Prefectures of Miyazaki, Ehime, Yamanashi, Nagano, Saitama and Ibaragi, while less severe in Tochigi, Shizuoka and also in Gumma.

The distribution of the disease will be shown in the following table and map based upon a recent survey conducted by the Department of Agriculture and Forestry of Japan (1929)[60] as well as by the writer.

As shown in Table 1 the extreme northern limits of the known distribution of the disease lie in the Prefecture of Miyagi. It does not coincide with the northern limits of the distribution of the leafhopper, *Nephotettix apicalis* var. *cincticeps* which transmits the disease, this insect having been found over a very wide area in Japan, extending into the Prefectures of Akita and Iwate for some distance, in the north. The leafhopper has been reported from practically every region in the southern prefectures.

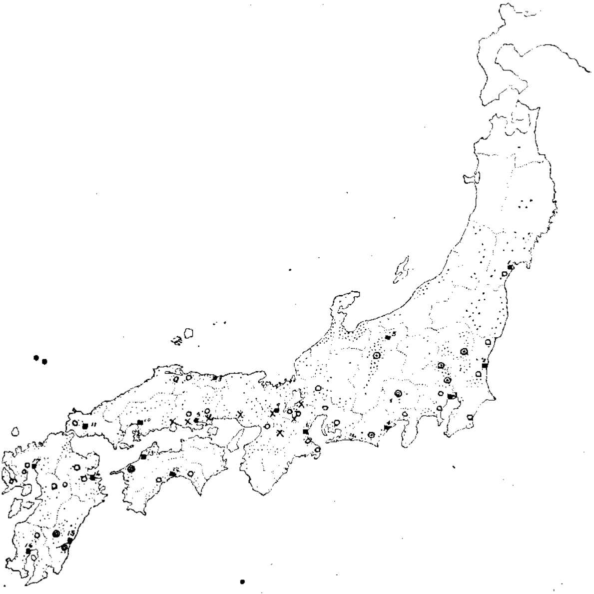
The leafhopper overwinters in its larval stage, usually feeding on *Astragalus sinicus* L. which is grown on rice fields through the winter and spring until it is ploughed in as a green manure in the early summer. In general it may be said that the dwarf disease of rice plant occurs in the regions where *Astragalus sinicus* is raised in abundance as will be seen in the following map.

TABLE 1. Geographical distribution of dwarf disease of rice plant

Locality	Amount of damage	Areas infested (in acres)	Historical remarks
Miyagi	slight	435	
Ibaragi	"	3750	*Severe in 1927
Tochigi	moderate	1250	
Gumma	"	—	Increasing since 1928, severe in 1929
Saitama	"	4100	
Chiba	slight	—	
Tôkyô	"	—	
Kanagawa	"	180	
Yamanashi	great	2500	Severe outbreaks in 1888–1889 and in 1915–1916
Nagano	moderate	5200	Severe in 1892
Shizuoka	"	500	*Severe in 1892 and 1893
Aichi	slight	—	
Gifu	"	—	
Mie	"	10	
Shiga	"	35	*Great ontbreak in 1892
Kyôto	nearly none	—	*Great outbreaks in 1889 and 1900
Ôsaka	slight	350	
Nara	nearly none	—	
Wakayama	"	—	
Hyôgo	"	—	Great outbreak in 1891
Okayama	slight	70	*Great outbreak in 1891
Hiroshima	nearly none	—	*Severe in 1902–1903
Yamaguchi	slight	70	First noted in 1910
Tottori	"	90	First noted in 1914 or 1915
Shimane	"	—	First noted in 1914
Ehime	great	3000	
Kôchi	slight	—	
Saga	"	—	
Nagasaki	"	—	
Ôita	"	—	
Miyazaki	great	137500	First noted in 1912 or 1913, great outbreak in 1930
Kumamoto	slight	500	*
Kagoshima	"	—	

(* The disease seems to have been known for a number of years though its origin is not definitely known)

Geographical distribution of dwarf disease of rice plant



Remarks

- • Localities where *Astragalus sinicus* L. is raised in abundance.
- ○ Localities where dwarf disease of rice plant occurs.
- ⊙ Localities where dwarf disease of rice plant is severe.
- ◎ Localities where dwarf disease of rice plant is very severe.
- × Localities where dwarf disease of rice plant was very severe.

1: Sendai, 2: Mito, 3: Tôkyo, 4: Shizuoka, 5: Nagano, 6: Tsu, 7: Kyôto, 8: Tottori, 9: Okayama, 10: Hiroshima, 11: Yamaguchi, 12: Kôchi, 13: Matsuyama, 14: Ôita, 15: Miyazaki, 16: Kagoshima, 17: Saga.

III. Review of literature

The earliest account of the dwarf disease of rice plant was given under the titile "Dwarfed rice plant, its cause and control measures."[92] in the Official Gazette of Oct. 18, 1890, by the Department of Education replying to the inquiry from the Government of the Prefecture of Kyôto. This article seems, as DAIKUHARA (1900)[39] cited it later, to have been written by KERNER, KOZAI and MORI of the Faculty of Agriculture of the Tôkyo Imperial University. It is mentioned in this report that the dwarf disease of rice plant is caused by the products of reduction induced in the soil. On account of the deficiency of oxygen in the soil, the sulphates contained in the soil and manure as well as in the irrigation-water are brought to reduction producing iron sulphide which inhibits the growth of rice plants. As the control measures it was recommended to oxidize the iron sulphide deposited in the soil by means of withdrawiug stagnant water, drying of the soil, application of unslaked lime and the surface firing of the soil.

In 1893 YAMADA* reported the results of analyses of the rice plants affected by the dwarf disease as compared with healthy plants, stating that the diseased plant just before the flowering time contains more crude fibre, iron oxide, calcium, sodium and sulphuric acid, and less crude fat, crude protein, non-nitrogenous extract, total nitrogen, albuminous nitrogen, non-albuminous nitrogen, magnesium, potassium, phosphoric acid, chlorine and silica than the healthy plant.

TAKATA[219] stated in 1895–96 that iron sulphide deposited in the soil, the amount of stagnant water, low temperatures of the irrigation water, climatic conditions or cultural practices do not account for the incidence of the disease but a certain species of leafhopper which he named "mon-yokobai" is responsible for the disease. He pointed out that the progeny grown from seed of diseased plants is not always affected with the disease and also that not all rice varieties are equally susceptible. The leafhopper which he called "mon-yokobai" is apparently *Deltocephalus dorsalis* MOTSCH. as shown by his description accompanied by illustrations. This leafhopper, however, has no connection with the disease as reported later by the Shiga Agr. Experiment Station. According to ISHIKAWA (1928),[86] HASHIMOTO in 1894–95 was the first to prove the causal relation of the leafhopper to the dwarf disease of rice plant. However, it is uncertain with what species of

* See (39)

leafhopper he worked.

The Shiga Agricultural Experiment Station undertook studies on the disease soon after its establishment in 1895 and continned the work during a period of 20 years. The results of earlier experiments have been reported in "Results of experiments with insect pests, Reports 1–8".[192] Some noteworthy points will be given below. In Report 1 (1897) it is stated that the dwarf disease of rice plant was caused by the leafhoppers, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL., *Deltocephalus dorsalis* MOTSCH., *Thamnotettix tobae* MATS., *Cicadula fasciifrons* STAL., and likewise by *Zygina limbata* MATS.? while none of the leafhoppers, *Delphacodes striatellus* FALL., *Tettigonia viridis* L. and *Nisia atrovenosa* LETH. was responsible for the incidence of the disease. It is also mentioned that the damage was markedly lessened by eliminating the leafhoppers by means of kerosene. It is evident that the disease was at this time entirely attributed to the leafhoppers. In Report 2 (1900) it is reported that only one species of all leafhoppers tested viz., *Nephotettix apicalis* var. *cincticeps* is capable of producing the trne dwarf disease of rice plant. It is also stated that new shoots from the affected plants always contracted the disease even though they had been kept free from the leafhoppers. In Report 6 (1906) it is stated that the leafhoppers collected in the Prefecture of Shiga were capable of producing the disease while those taken from Tôkyo were uninfective. This finding was confirmed in Reports 7 and 8 (1908) showing that the leafhoppers taken from the Prefecture of Okayama produced the disease as well as those from the Prefecture of Shiga but that the leafhoppers from Tôkyo and the northern districts of Honshû were entirely uninfective. Furthermore it is stated that uninfective leafhoppers taken from Tôkyo or the northern districts of Honshû became able to induce the disease if they fed on the dwarf diseased rice plant for about 5 days. Thus it became evident that the leafhopper itself does not cause the disease but transmits it acting as the vector. This was first shown by the experiments performed in 1906 and confirmed during the following years. Accordingly it is in 1908 that the leafhopper, *Nephotettix apicalis* var. *cinticeps* was first authentically recorded to transmit the dwarf disease of rice plant, although the causal relation of the leafhopper to the disease had been proved 8 years before. The results of the experiments carried out by the Shiga Agricultural Experiment Station from 1895 to 1908 have been summarized in its Special Bulletins No. 1 and 2,[103] issued in 1906 and 1910 respectively.

TAKAMI (1901)[218] also summarized the results of the earlier experiments of this exp. station, ascribing the disease to the infestation of the leafhopper. The results of the later experiments have been recorded in the Annual Reports for 1907 to 1915,[194] the following points being worthy of note. Besides the rice plant, it is stated, *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA was affected by the disease but that neither *Setaria italica* BEAUV. nor *Panicum miliaceum* L. was subject to the disease. It is also mentioned that a variety of rice plant, "Ishuku-shirazu" was immune from the disease and that a single infective leafhopper was capable of transmitting the disease to a healthy plant when fed on it for only one day.

Either before or after the work of the Shiga Agricultural Experiment Station, the problem of the dwarf disease of rice plant was also attacked by the Agricultural Experiment Stations of Hyôgo (1895–9),[84] Nara (1897),[153] Okayama (1902–9),[159] Hiroshima (1903)[77] and Kyôto (1905, 1910).[234, 117] They reached the same conclusion as regards the causal relation of the leafhopper to the disease.

Meanwhile, DAIKUHARA (1902, 1904)[39] reported the results of biochemical studies on the dwarf disease of rice plant. According to him the affected leaves contain more crude proteins and ashes, and less crude fibre, crude fat and non-nitrogenous extract than the healthy leaves. They contain much more phosphoric acid, potassium and magnesium, and relatively less calcium than the normal leaves do. The rate of transpiration as well as the translocation of starch is remarkably decreased in the leaves of affected plants. The amount of oxidizing enzymes, particularly peroxidase, and diastase is greater in the affected leaves as compared to that in the healthy leaves while catalase is less active in the former. Mechanical injuries given to the leaves and roots, sorts of manure, and cultural practices do not account for the incidence of the disease. Transmission was not effected by injection of juice from the diseased plant into healthy seedlings. Not all rice varieties are equally susceptible to the disease, "Ishuku-shirazu" being the most resistant. After all DAIKUHARA considered the dwarf disease of rice plant as a disease allied to the sereh of sugar cane, the moasic disease of tobacco and likewise to the stigmonose of carnations.

In 1905 YONEMARU and ASO[234] reported the results of their experiments in connection with the disease in a Special Bulletin of the Kyôto Agricultural Experiment Station. They also stated that the diseased leaves contained more nitrogen, particularly amides, and much more

oxidizing enzymes than the healthy leaves, and that the seedlings derived from the seed of diseased rice plants did not necessarily contract the dwarf disease. They mentioned that the injury due to leafhoppers could not be considered as the main cause of the dwarf disease but *Nephotettix apicalis* var. *cincticeps* tended to induce the disease. The outline of this report has been published in the Extra Bulletin No. 12[117] of the same experiment station, which was issued in 1910.

In 1910 Andô[7] briefly reported the results of transmission experiments which had been carried out in the Imperial Agricultural Experiment Station. In 1905, according to him, the leafhoppers from Tôkyo were allowed to feed upon dwarf diseased rice plants taken from Shiga Prefecture and then the insects of the second or third generation proved to be capable of producing infections in healthy plants. He further stated that the uninfective leafhoppers from Tôkyo became infective when allowed to feed upon diseased rice plants for 15 days and that the infective power was not transmitted to the progeny, when the infective parents were transferred to healthy rice plants at 5–7 days' intervals in order to keep them from sucking the juice of diseased plants. He emphasized that the disease is not caused by the leafhopper but by certain unknown infective principle which is carried by the leafhopper.

During the Taisho Era (1912–1926) the disease has been investigated by the Agricultural Experiment Stations of Shiga,[194] Kagoshima,[97] Miyazaki,[144, 145] Nagano[152] and Yamanashi,[233] establishing the causal relation of the leafhopper, *Nephotettix apicalis* var. *cincticeps* to the disease.

Prior to this, the Imperial Agricultural Experiment Station in Nishigahara near Tôkyo and its Kyûshû Branch Station in Kumamoto had taken up studies on the disease since the turn of the century as cited by Bokura (1930)[21] and also by Murata (1931).[149] The results of investigations have been reported giving only brief notes in the Annual Reports of the Experiment Station[85] during the Taisho Era. In the Annual Report for 1913–1915 issued in 1916 it is stated that the presence of certain protozoa was noticed in the tissues of the diseased rice plants as well as in the blood of the leafhoppers. However, no detailed account has been given of these protozoa.

Kasai[99] reported in 1924, the finding of Nelson's bodies in association with the dwarf disease of rice plant and several other virus diseases of plants, stating that these bodies represent no protozoa but the degenerated or even normal nuclei of the host plant. However, as shown

by his descriptions and illustrations these bodies are apparently different from those described and figured by NELSON (1922).[156]

Recently the writer (1931)[68] reported that certain intracellular bodies are invariably present in the chlorotic tissue of the leaf of the rice plant affected by the dwarf disease.

IV. Symptoms of the disease

The dwarf disease of rice plant usually appears in late June when the rice plants have been transplanted into the rice field and grown 6–8 inches high, although it is not unusual to find the disease in the seed bed.

The first visible symptom of the disease manifests itself as yellowish white specks along the veins of newly unfolded leaves. These specks which develop before the leaves unfold, are yellowish green to yellow when viewed by diffused light. By holding up to the light these specks become distinct, being yellowish white to white in color. The specks elongate and spread out along the leaf parallel to the midrib, forming fine interrupted streaks. (Pl. II, Fig. 4). These range from mere dots to an area several millimetres in length and from 0.2 to 1 mm. in width. The succeeding leaves invariably show the white specks while the lower, previously formed leaves exhibit no signs of the disease. On the leaf which shows the first visible symptom of the disease the specks may be confined to the lower part of the leaf blade or to only one side of the midrib near the base of the leaf. On the succeeding leaves more conspicuous specks develop in abundance and connect with each others forming almost continuous streaks along the veins. Thus the symptom of the disease becomes rather conspicuous in the middle of July.

The growth subsequent to infection being much arrested, the diseased plant becomes remarkably stunted, and the internodes shortened, while numerous diminutive tillers develop, producing a rosette appearance. Affected plants tend to develop a foliage dark green in color.

The roots of the affected plants are much arrested in their growth, and only small roots develop extending horizontally. This condition becomes especially evident when such a plant is transplanted.

The plants infected in their very early stage of growth, become severely stunted being at most 10 inches high. (Pl. I, Fig. 1). They produce none or only a few worthless panicles. The symptoms of the plants affected at a later stage of their development are less prominent.

Upon one or two of the younger shoots of such a plant, the streak may appear but the other shoots remain entirely healthy.

V. Intracellular bodies associated with the disease

1. *Occurrence of intracellular bodies in the affected rice plants*

As stated in the writer's previous paper,[68] certain intracellular bodies were revealed both in fresh and embedded material of leaves of rice plant affected with dwarf disease. Similar bodies were found also in the crown and root of the diseased plant.

For fixation of the material several fixatives were used including medium chromo-acetic solution, FLEMMING's fluid, MERKEL's fluid, ALLEN's modification of BOUIN's fluid, GILSON's fluid, ZENKER's fluid, acetic alcohol, formol-acetic alcohol and SCHAUDINN's fluid. Among these, chromo-acetic solution and formol-acetic alcohol proved to be most suitable for general purposes. The fixed material was embedded in paraffin and sectioned by means of a microtome in the usual way. Transverse and longitudinal sections were cut 5 microns thick and stained with HEIDENHAIN's iron-alum haematoxylin or safranin and gentian violet. Longitudinal sections were preferable to demonstrate the intracellular bodies. Some of the materials which had been fixed in SCHAUDINN's fluid, GILSON's fluid, ZENKER's fluid and formol-acetic alcohol were stained with GIEMSA's stains. The GILSON-GIEMSA combination gave very satisfactory results. Attempts were also made to demonstrate chondriosomes, if any, in the intracellular bodies, by fixing the material in REGAUD's fluid, BENDA's fluid, CHAMPY's fluid and SAPĚHIN's fluid and staining the sections with iron alum haematoxylin or with ALTMANN's acid fuchsin.

Microscopic studies of free hand sections of the diseased leaf show chlorotic modifications in the mesophyll-cells adjacent to some of the vascular bundles. In sections mounted in water, the chlorotic tissues are lighter in color or nearly colorless, the chloroplasts in these cells being light colored and smaller in size and number, due to their disintegration. In the cell where most of the chloroplasts have been disintegrated, a certain foreign body is found in close proximity to the nucleus. Such foreign bodies are usually round in shape, more or less larger than the host nucleus and they contain many vacuoles of varying size. These bodies stain yellow in dilute iodine potassium iodide solution, brick red in aceto-carmin, Grenadine pink (after RIDGWAY[173]) in

the alcoholic solution of Sudan III, and pinkish cinnamon in MILLON's reagent.

However, more comprehensive studies of the intracellular bodies can be done in the microtomed sections properly stained. In the preparations stained with HEIDENHAIN's haematoxylin, the chlorotic areas are easily recognized since the chloroplasts in these tissues show less affinity for the stain, and they are, according to the degree of chlorosis, smaller and less regular in shape and fewer in number than those in the apparently healthy tissues of the same leaf (Pl. III, Fig. 11). Haematoxylin brings out the intracellular bodies very sharply, since they take the stain as intensely as the host nuclei. When sections are stained with safranin and gentian violet, the bodies show a special affinity for safranin while gentian violet stains the host nuclei violet in color. In the sections stained with GIEMSA's stains the intracellular bodies show deep blue ("sailor blue" of RIDGWAY) whereas the host nuclei stain purple to violet ("mallow purple" to "nigrosin violet") and the chloroplasts light lilac. The foreign bodies under consideration occur singly in the host cells but occasionally two of them are found in the same cell. They are more abundant in the mesophyll cells but it is not unusual to find them in the epidermal cells (Pl. III, Fig. 8, 9, 12, 13). The bodies are located near or in close contact with the host nucleus which frequently becomes much enlarged. They vary considerably in shape and size. Round to oval are the most usual forms but it is not uncommon to find bodies amoeboid or very irregular in shape. In size they range from 3 to 10 microns in length and 2.5 to 8.5 microns in width, frequently much larger than the host nuclei which are 2.5 to 3.5 microns in diameter. The contents of the intracellular bodies appear to consist of a somewhat homogeneous structure, surrounded by an indistinct membrane, and contain many vacuoles of various size (Pl. IV, Fig. 14, 15, 16). The vacuoles seemed more conspicuous in the bodies from the material fixed in formol-acetic alcohol or ZENKER's fluid. All attempts to demonstrate a nucleus or chromatin granules in these bodies were unsuccessful but minute granules resembling the so-called chlamydozoa were revealed, although their exact nature was not definitely ascertained (Pl. III, Fig. 12, 13). A search for chondriosomes in these bodies by use of recommended fixatives and stains revealed no bodies of this type. Although the intracellular bodies occur invariably in the chlorotic areas of the leaf, as yet they have not been found in the intact tissue of the diseased leaf or in plants free from the disease. Quite similar bodies have been demon-

strated in the phloem and its adjacent tissues in the root (Pl. III, Fig. 10) and crown of the diseased plant. They are found abundantly in the parenchyma-cells, near or in close contact with the host nuclei.

The intracellular bodies in question are not artifacts, since they can be found in the cells of fresh material as well as in material fixed by various methods and stained with various stains. It it also evident that these bodies are neither deformed or degenerating nuclei, nor hypertrophied chloroplasts of the host cells, although their exact nature remains still unknown. Beyond doubt they are similar to the intracellular bodies which have been found in association with certain virus diseases of plants and animals.

2. *Occurrence of the intracellular bodies in the other susceptible plants*

As will be described more fully later, *Panicum miliaceum* L., *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA and *Alopecurus fulvus* L. are readily affected by dwarf disease of rice plant whereas wheat, rye and oats are more or less susceptible to the disease. It seemed advisable, therefore, to examine the chlorotic tissues of the affected leaves of these plants in the hope of finding the intracellular bodies which have been demonstrated in diseased rice plants. The affected leaves were fixed in GILSON's fluid and embedded in paraffin in the usual way. Longitudinal and transverse sections were cut 7 microns thick and stained with GIEMSA's stains. Certain foreign bodies have been found to occur invariably in the chlorotic tissues of leaves of the above mentioned plants with the exception of oats which were not studied in this connection owing to the defficiency of suitable materials. These bodies agree in every respect with those found in the leaves of diseased rice plants (Pl. IV, Fig. 17, 18, 19). They are localized usually in the tissues adjacent to the vascular bundles and near or in close contact with the host nuclei which are more or less smaller than them. The shape, structure and staining reaction of these bodies very closely resemble those described in respect of the intracellular bodies found in rice plants and repetition here would be superfluous.

3. *Previous investigations of the intracellular bodies associated with virus diseases of plants*

IWANOWSKI (1903)[89] was the first to discover the intracellular bodies in association with virus diseases of plants. Working with the mosaic

disease of tobacco, he found in the cells of chlorotic leaf tissues certain amoeboid plasmic aggregates which occur near or in close contact with the nuclei. He considered that these amoeboid bodies were reaction products of the cells resulting from the irritation by the parasite and that they could not be a causal organism of the disease because bodies as large as these could not possibly pass through a bacterial filter. IWANOWSKI found also the presence of granular cell-inclusions which he thought to be zoogloea of bacteria, the causative agent of the mosaic disease. However he could not successfully culture the bacteria which would cause the mosaic disease. The findings of IWANOWSKI were confirmed by PALM (1922),[161] who agreed with the former that the amoeboid bodies in the diseased cells were the result of reaction of the cell to the irritation of the causative agent. PALM, however, considered that the minute granules which IWANOWSKI had assumed to be bacteria "agreed *in every respect with the Strongyloplasmen of* LIPSCHÜTZ" *or the* chlamydozoa of PROWAZEK, being homologous with the GUARNIERI bodies. He believed that these small granules might be the causal agency of the mosaic disease of tobacco and named them *Strongyloplasma iwanowskii*.

The intracellular bodies associated with mosaic disease of tobacco were further studied by DICKSON (1925),[44] GOLDSTEIN (1924, 1926),[72,73] KUNKEL (1924),[110] RAWLINS and JOHNSON (1925),[170] F. F. SMITH (1926),[196] HOGGAN (1927),[78] KLEBAHN (1928)[104] and LIKHITÉ (1929).[119] Notably GOLDSTEIN studied the vacuolate amoeboid bodies which she called X-bodies, in the living condition and noticed an apparent change in form and their "distinct amoeboid movement", coming to believe that these bodies are the causal organisms of mosaic disease. LIKHITÉ also regarded the X-bodies as representing a living parasitic organism for which he proposed the name *Vacuolarium ivanowski*. HOGGAN, on the other hand, presented some evidence to show that the X-bodies are products of diseased cells but not of the nature of a causal organism.

The occurrence of similar vacuolated bodies has been shown in mosaic diseases of tomato (HOGGAN 1927),[78] pepper (HOGGAN 1927),[78] potato (K. M. SMITH 1924,[200] HOGGAN 1927,[78] CLINCH 1932[36]), petunia (F. F. SMITH 1926,[196] HOGGAN 1927[78]), Datura (F. F. SMITH 1926)[196] and some other solanaceous plants (HOGGAN 1927,[78] J. H. SMITH 1930,[198] SHEFFIELD 1931[190]); likewise in corn mosaic (KUNKEL 1921),[105] sugar cane mosaic (KUNKEL 1924,[110] COOK 1925[38]), wheat mosaic (McKINNEY et al. 1923),[137] Chinese cabbage mosaic (KUNKEL 1924),[110] Hippeastrum mosaic (KUNKEL 1922,[106] 1924,[110] McKINNEY et al. 1923,[138] HOLMES

1928),[82] Phytolacca mosaic (F. F. SMITH 1926),[196] Dahlia mosaic and dwarf (GOLDSTEIN 1927),[74] "infectious chlorosis" of *Euonymus japonicus* (F. F. SMITH 1926),[196] Fiji-disease of sugar cane (LYON 1910,[122] 1921,[123] REINKING 1921,[172] McWHORSTER 1922,[142] KUNKEL 1924[109]), spike disease of sandal and *Vinca rosea* L. (NARASIMHAN 1928,[154] 1933[155]), lily mosaic (GOLDSTEIN 1928)[237] and in curl of the vine (PETRI 1931).[162]

As to the nature of these bodies there exists a remarkable difference in opinion. LYON (1921) who first reported the presence of such bodies in the galls produced on the sugar cane leaves affected with Fiji disease looked upon them as parasitic organisms and proposed the name *Northiella sacchari* although its systematic position was not definitely known. KUNKEL (1924) also considered these bodies to belong to a parasitic organism, being capable of division and appearing to grow. McWHORSTER (1922) stated that the foreign body present in the cells of Fiji galls was an amoeba which could be cultured in cane juice and he named the organism *Phytamoeba sacchari*. KUNKEL (1921, 1924), giving a detailed account of similar intracellular bodies associated with the mosaic diseases of corn, *Hippeastrum equestre*, *Brassica pekinensis* and sugar cane, stated that these bodies might represent "one stage in the life of a causal organism". HOLMES (1928) working with the intracellular bodies in Hippeastrum mosaic came to the conclusion that these bodies consist of living cytoplasm, chondriosomes being found within the bodies. He states: "Whether the body represents a stage in a foreign organism, a mass of plant cell cytoplasm containing virus, or a mass of the cell cytoplasm not immediately in contact with virus but stimulated by the diseased condition, is not known." K. M. SMITH (1924) found similar amoeboid bodies in the chlorotic leaf tissues of mosaic diseased potato plants, which he considered to be some degeneration product of the cell induced by the mosaic, being effects rather than causes of the disease. HOGGAN (1927) found X-bodies consistently in different solanaceous plants affected with tobacco mosaic or yellow tobacco mosaic etc., but no such bodies could be demonstrated in the same plants when affected with cucumber mosaic or other virus diseases. She also found "vacuolate protoplasm-like material" in the cells of potato in association with several different virus diseases, appearing in various forms, either as definite bodies or as irregular masses whose appearance suggested intermediate stages between the bodies and the host cytoplasm. Accordingly she came to the conclusion that the X-bodies were not of

the nature of a causal organism. CLINCH (1932) also found similar vacuolate bodies in association with simple mosaic, interveinal mosaic, crinkle, and streak of potato. According to him mitochondria are present in these bodies and numerous fatty globules are associated with the X-bodies at certain stages. He suggested that the X-bodies arise as a result of viscosity changes in the cytoplasm of the diseased cells. BREHMER and BÄRNER (1930)[31] considered the amoeba-like bodies associated with virus diseases of potato to be the causal fungus which belongs to the genus Plasmodiophora.

GOLDSTEIN (1927) claims that she was able to demonstrate nucleus-like structures within the X-bodies in the cells of dahlia plants affected by mosaic and dwarf disease. She wrote that the X-bodies could not be considered "simple degeneration products of the host cells, or cell nucleus, because of their very protoplasmic appearance and structure, and the presence within them of internal vacuoles and nucleus-like structures, the presence of peripheral vacuoles, and their apparent division by constriction".

The other investigators did not attempt to interpret the significance of intracellular bodies which they found.

According to MCKINNEY (1925),[134] there are two types of intracellular bodies in association with virus diseases of plants. The first type is, according to him, represented by the intracellular bodies associated with tobacco and potato mosaics, the Fiji disease of sugar cane and mosaic disease of wheat, which resemble each other in form and structure. Most of the bodies possess a matrix which is homologous in nature and contains vacuoles varying in number and size. All of these bodies appear to be surrounded by a membrane and to consist of protoplasm. The intracellular bodies associated with the mosaic diseases of corn, Hippeastrum, and sugar cane represent the second type, presenting a somewhat different appearance from the above mentioned bodies. Most of these bodies seem, MCKINNEY writes, to be made up of compactly arranged rather large, independent granules and not to be surrounded by a membrane, and they are not so suggestive of protoplasm as the bodies of the first type. HOLMES (1928), however, claims that the intracellular bodies associated with Hippeastrum mosaic consist of living protoplasm as mentioned before.

It appears that the intracellular bodies which the writer found in association with dwarf disease of rice plant belong to the first type, if there really do exist two types of intracellular bodies as pointed out by

McKINNEY.

Several other types of cell inclusions have been described as associated with certain virus diseases of plants. Among these are plasmodium-like substance in the mosaic diseased cane leaf and stem tissues found by MATZ (1919[126]); "flagellated organisms" associated with mosaic of tomato, dahlia, squash, and Hippeastrum, which were described by ECKERSON (1926[56]); the minute "hyaline bodies" reported by SOROKIN (1927)[204] to occur in the transparent spheres resulting from the destroyed chloroplasts in the mosaic diseased tomato leaf; KLEBAHN's scolecosoma (1926,[103] 1928[104]) and other cell inclusions in association with "Alloiophyllie" of *Anemone nemorosa*, "infectious chlorosis" of *Abutilon thompsoni*, and mosaic diseases of tobacco and potato etc.; similar bodies in dahlia and lettuce mosaic (BRANDENBURG 1928);[236] SCHAFFNIT-WEBER's elytrosoma associated with mosaic diseases of beet and broad bean (1927[178]); "amorphous bodies" reported by PLAKIDAS (1927,[164] 1928[165]) to occur in the root tissues of straw-berry affected with yellows or dwarf, and similar structures demonstrated by RAWLINS (1926)[169] in the root tips of sugar beets affected with curly top.

No attempt will be made to discuss in detail these inclusion bodies because it seems to the writer that such bodies do not fall in the same category with the intracellular bodies associated with dwarf disease of rice plant and moreover, some of these bodies have been found to occur in healthy plants.

4. *Absence of inclusion bodies in the viruliferous leafhopper*

Because of the occurrence of the so-called chlamydozoa-like bodies in the intracellular bodies associated with dwarf disease of rice plant, it seemed advisable to look for similar bodies in the viruliferous leafhopper. Such a study might result in the discovery of the causative agent of the disease or some indication of the presence of the virus.

The infective leafhoppers, both nymphs and adults, were fixed in GILSON's fluid and embedded in paraffin in the usual way. Transverse and longitudinal sections were cut 5 or 7 microns thick and stained with GIEMSA's or MALLORY's stains. Similar virus free leafhopprs served as checks.

In certain insect borne diseases of milkweeds (HOLMES 1925)[81] and Euphorbias (FRANÇA 1914)[63] caused by protozoans, the causal organisms have been found in the salivary glands and in the alimentary canal of

their insect carriers. Accordingly it seemed desirable to inspect particularly these organs of the leafhopper in the hope of finding the causative agents or some indication of the presence of the virus. As will be more fully stated later, the virus may be transmitted from the viruliferous females to their offspring through the eggs which are in all probability infected by the virus at an early stage of development in the ovaries of the maternal insects. The ovaries, therefore, must not be left out of consideration.

The alimentary canal consists of pharynx, oesophagus, ventriculus, intestines and rectum. The pharynx is represented by a flattened aperture, at the proximal end of which the oesophagus commences, passing through the cephalic ganglion. The oesophagus then extends into the thorax where it opens into the ventriculus. The oesophagus wall is composed of flat epitherial cells and is lined with a cuticular intima. The ventriculus is narrow in front and widest in the posterior region of the thorax where it passes into the abdomen to be connected with the midintestine. The digestive epithelium of the ventriculus consists of large cells which project into the lumen while the walls of the mid-intestine are lined with an epithelium of large columnar cells. Malpighian tubules arise at the point of junction of the mid- and distal-intestines. The tubules are four in nnmber, very long and convoluted. They have a moniliform appearance and are of uniform width throughout. Both the intestines and Malpighian tubules are intimately bound up with the diffuse fat bodies (Pl. V, Fig. 26).

The salivary glands lie in the prothorax at both sides of the oesophagus. They are composed of a number of glandular cells which are somewhat triangular in section and possess considerably large nuclei (Pl. V, Fig. 22 & 24).

The ovaries of an adult leafhopper occupy the dorsal and lateral regions of the abdominal cavity. The ovarian wall is very thin and each ovarian tubule is filled with numerous egg follicles in various stages of development. The egg cell is round in shape and enveloped by the follicle epithelium which is uniform in thickness and composed of cuboidal cells (Pl. VI, Fig. 27).

The mycetome or pseudovitellus is an organ present in most *Homoptera*. It is a large paired organ located in the first three abdominal segments and has no direct connection with any other organs. The cells composing it harbour microörganisms of a fungal nature and accordingly are termed myeetocytes. It is generally believed that these

microörganisms are symbiotic fungi and this subject, along with their origin and incidence in different insects is discussed by BUCHNER (1930).[32] In all the *Homoptera* the symbionts are transmitted from one generation to the next through the eggs. The mycetome itself has no direct connection with dwarf disease of rice plant because it is present in virus-free as well as in viruliferous leafhoppers. But since both the mycetome and virus have been known to be transmitted from one generation to the next through the eggs, it seemed desirable to look for some indication of the presence of the virus in the mycetome. The mycetome of the leafhopper under consideration is composed of three distinct zones, comprising different types of cells (Pl. VI, Fig. 28). The central portion is occupied by numerous compact angular cells which are provided with large nuclei and packed with mycelium-like strands. Sometimes the inner cells appear to have collapsed, leaving the nuclei among clumps of cytoplasm (Pl. VI, Fig. 29). The next zone is composed of cells, whose outlines are obscure owing to the presence of thick mycelium-like strands which fill up the cell lumens. The nuclei, however, can be found among these mycelium like strands. The third zone is very thin, being one cell thick, and borders the mycetome. These outer cells are small and flat.

In spite of a careful examination of thousands of both transverse and longitudinal sections of viruliferous leafhoppers, the writer was unable to find any inclusion bodies or microorganisms of etiological significance, nor visual evidence of the presence of the virus in the salivary glands and the alimentary canal, in the egg follicles in the ovarian tubules, nor likewise in the mycetome and other organs.

DOBROSCKY (1929),[46] working with aster yellows, also could not find any bacterium, Rickettsia, fungus, protozoan, or X-body, of etiological significance, or visual evidence of the presence of the virus in the leafhopper, *Cicadula sexnotata* FALL. According to SWEZY and SEVERIN (1930),[217] the beet leafhopper, *Eutettix tenella* BAKER, the carrier of curly top of sugar beets, harbors in its alimentary tract and blood two different bacilliform organisms, which "possess some of the characteristics of Rickettsia". However, the causal relatoins of these organisms to curly top of beets has not been established.

VI. Results of transmission experiments

A) No transmission through seeds

The rice plants affected with the dwarf disease at a later stage of

their development often produce a few small panicles with some matured grains, even though the plants as a whole become markedly stunted.

In the fall of 1922, about 200 seeds were collected from diseased rice plants in a certain locality in Tottori Prefecture. On May 8, 1923, these seeds were sown in 7 Wagner pots, after they had been soaked in water for a week. On July 4 the resulting seedling plants were transplanted, taking each 3 plants together into a pot. The plants were not protected in any way since the dwarf disease of rice plant has never been noticed in the vicinity of the City of Tottori where the writer's experiments were carried out. The total number of plants grown from seeds of diseased plants was 165, that is, 135 of "Aikoku" and 30 of "Hayakitabu", both varieties being very susceptible to the dwarf disease. None of these plants developed any symptoms of the disease throughout the season.

On May 28, 1929, 420 seeds of "Aikoku"-variety which had been collected in the previous autumn from diseased plants were sown in several pots. Three hundred and twenty eight plants were grown from these seeds. All these plants remained healthy during the 3 months that they were kept under observation.

As shown above, 493 rice plants in all were grown from seeds of diseased plants and in no case did an affected plant appear.

TAKATA (1895–96)[219] and YONEMARU and ASO (1905)[234] stated that the seedlings grown from the seed of dwarf diseased rice plants do not necessarily contract the dwarf disease. Similar results were also reported in the Report of the Okayama Agricultural Experiment Station published in 1902.

It seems to be conclusive that the dwarf disease of rice plant is not transmitted through seeds.

Most virus diseases of plants are not readily transmitted through seeds. This has been reported true by various investigators regarding these diseases: tobacco mosaic (MAYER 1886,[124] ALLARD 1912,[1] 1914,[2] 1915,[3] CLINTON 1915[37]), tomato mosaic (ALLARD 1916,[4] GARDNER and KENDRICK 1922,[71] DICKSON 1922[42]), sugar cane mosaic (WILBRINK and LEDEBOER 1910[229] beet mosaic (LIND 1915,[120] ROBBINS 1921,[174] BÖNING 1927,[25] JONES 1931[96]), spinach blight (McCLINTOCK and SMITH 1918),[129] potato mosaic (SCHULTZ and FOLSOM 1923),[182] corn mosaic (BRANDES 1920,[28] BRANDES and KLAPHAAK 1923[80]), crucifer mosaic (CLAYTON 1930),[35] peach yellows (SMITH 1888,[195] BLAKE, COOK, and CONNORS 1921[19]), curly top of beet (SHAW 1910,[189] SEVERIN 1921[186]),

ster yellows (KUNKEL 1926),[111] streak of maize (STOREY 1925),[210] rawberry yellows (PLAKIDAS 1927),[164] rosette of peanuts (STOREY and BOTTOMLEY 1928),[215] mosaic disease of *Prunella vulgaris* (LIRO 930),[121] and leaf curl of cotton (KIRKPATRICK 1931).[102]

On the other hand, mosaic diseases of legumes and certain other virus diseases of plants have been reported to be seed-borne. They are bean mosaic (REDDICK and STEWART 1919,[171] ARCHIBALD 1921,[8] KURIBAYASHI 1926,[114] MEULEN 1928,[143] PIERCE and HUNGERFORD 1929,[163] MERKEL 1929,[142] FAJARDO 1928,[59] 1930[60]), soy been mosaic (GARDNER and KENDRICK 1921,[69] 1924[166]), lima bean mosaic (McCLINTOCK 1917),[127] Adzuki bean mosaic (MATSUMOTO 1922),[125] lettuce mosaic (NEWHALL 1923),[157] cucumber mosaic (DOOLITTLE and GILBERT 1919,[51] DOOLITTLE 1920,[49] DOOLITTLE and WALKER 1925,[54] BEWLEY and CORBETT 1930[18]), leaf roll of potato (MURPHY and McKAY 1924,[151] ELZE 1931[57]), tobacco ring spot (VALLEAU 1930,[222] 1932,[223, 224] HENDERSON 1931[75]), and Aucuba mosaic of potato (ELZE 1931).[57] According to DOOLITTLE and WALKER (1925)[54] cucumber mosaic is not transmitted through the seeds of cucumber, muskmelon, squash and pumpkin, but is transmitted through the seeds of *Micrampelis lobata*. BEWLEY and CORBETT (1930),[18] however, claim that cucumber mosaic is transmitted through cucumber seeds.

As regards the mosaic diseases of broad beans (BÖNING 1927,[24] MEULEN 1928,[143] MERKEL 1929,[142] FUKUSHI 1930[67]), peas (DOOLITTLE and JONES 1925,[52] MEULEN 1928,[143] MERKEL 1929[142]) and clovers (MEULEN 1928,[143] MERKEL 1929[142]) evidence has been offered to indicate that they are not seed-borne, although certain workers (DICKSON 1922,[42] 1924,[43] DICKSON and McROSTIE 1922[45]) claimed that mosaic diseases of peas and clovers were transmitted through the seed. The seed transmission of the tomato mosaic is also a disputed point. WESTERDIJK (1910)[228] and likewise BEWLEY and CORBETT (1930)[18] claimed that it was seed borne while ALLARD (1916),[4] GARDNER and KENDRICK (1922)[71] and DICKSON (1922)[42] stated that the mosaic was not transmitted through tomato seeds.

B) No Transmission through the Soil

It is not infrequent to find in the rice fields that the rice plants grown in close proximity to the dwarf diseased plants are entirely free from the disease. Moreover it has often been noticed that some of the

potted rice plants remained healthy in spite of their roots being intermingled with those of the other plants in the same pot, which have contracted the disease.

These observations seem to indicate that the causal agency of the disease is not soil-borne. In order to obtain further evidence the following experiments were carried out.

July 19, 1929, 8 diseased plants were planted into as many Wagner pots and in each of them 5 seedling plants were planted around the diseased plant. These seedlings were 3 weeks old at this time. None of these 40 plants developed the symptoms throughout the season, although their roots intermingled with those of the diseased plants.

August 20, 1931, 10 porcelain pots, 6 inches in diameter and 6 inches in depth, were filled with infested soil and remains of diseased plants. In each pot was planted one diseased plant. Five of these pots received each 10 two-week-old rice seedlings, which were transplanted around the diseased plant, while the other five pots likewise received each 10 rice seeds sown around the affected plant. Consequently 100 resulting plants were grown in close proximity to the diseased plants. A month later a small wood slip was thrust to a considerable depth at several points into the soil in the pots in order to injure some portion of the root system, which might furnish the entrance of infection. All of these 100 plants remained healthy coming into flower.

The results of these experiments are considered to be additional evidence against the transmission of the disease through the soil.

Most virus diseases of plants are not transmitted through the soil. With the following diseases, there is no evidence in favor of soil transmission: potato leafroll (WORTLEY 1918,[232] BOTJES 1920,[26] MURPHY 1921,[150] KASAI 1921,[98] SCHULTZ and FOLSOM 1921[181]), potato mosaic (SCHULTZ and FOLSOM 1920),[180] potato streak (ATANASOFF 1922),[9] sugar cane mosaic (BRANDES 1919),[27] peach rosette (McCLINTOCK 1923),[128] curly top of sugar beets (SEVERIN 1924),[187] broad bean mosaic (BÖNING 1927),[24] beet mosaic (BÖNING 1927),[25] spinach blight (McCLINTOCK and SMITH 1918),[129] cucumber mosaic (DOOLITTLE 1920,[49] DOOLITTLE and WALKER 1925[54]), rosette of peanuts (STOREY and BOTTOMLEY 1928),[215] leaf curl of cotton (KIRKPATRICK 1931)[102] and others.

Certain virus diseases, however, have been stated to be transmitted through the soil. Mosaic disease of wheat and rye has been proved to be so transmitted. (McKINNEY 1923,[132] 1925[133]; WEBB 1928[227]).

Mosaic disease of tobacco also has been reported by certain workers to be transmissible through the soil (MAYER 1888,[238] BEIJERINCK 1898,[17] CLINTON 1915,[37] MCKINNEY and WEBB 1926,[140] MCKINNEY 1927,[136] MOUTIA 1928,[146] JOHNSON and OGDEN 1929[95]). In this case, the soil infection appears to depend upon injury to some portion of the root system which happens to come in contact with the infected material. DOOLITTLE (1928)[50] claims the soil transmission of tomato mosaic and streak in the green house.

C) Attempted transmission with the juice of diseased plant

DAIKUHARA (1904)[39] was the first to attempt the transmission of the dwarf disease of rice plant with the juice from the diseased plant. No transmission was obtained by hypodermic injection of the expressed sap from the diseased plant into the veins of the leaves of seedlings. In the Annual Report of the Imperial Agricultural Experiment Station for the year 1912,[85] it is stated that all attempts to transmit the disease with the juice from the diseased leaves or the ground up bodies of leaf-hoppers have failed.

The results of the writer's experiments along the same line also tend to substantiate the view that the dwarf disease of rice plant cannot be transmitted by mechanical inoculations.

Experiment 1. On August 15, 1923, 19 three-week-old seedlings of rice plant ("Aikoku"-variety) were artificially inoculated by hypodermic injection of the expressed juice from the diseased plant. Each seedling received 0.5 cc. of the inoculum which was injected into the stem near the ground. All the inoculated plants remained healthy throughout the season.

Experiment 2. October 4, 1923, 24 seedlings of the "Aikoku"-variety were inoculated in the same manner as above. These seedlings were also about three weeks old. Each seedling received 1 cc. of the inoculum which had been prepared grinding fresh diseased leaves in a mortar, adding a small quantity of distilled water, until the leaf tissue was thoroughly crushed. No symptoms of the disease appeared on any of the inoculated plants.

Experment 3. August 8, 1927, 15 rice plants were inoculated by means of the needle puncture method. For the preparation of the inoculum, three diseased plants were cut into pieces and ground in a mortar, adding a small quantity of water until the tissue was thoroughly

crushed. Three inoculations were made on each plant, one near the growing tip, another at the node and the other at the base of the stem. A large drop of the inoculum (about 0.5 cc.) was placed with a sterile scalpel on the desired spot of the stem, and with a sterile needle about 30 pricks were made through the fluid, thus working the latter into the tissue. In no case did the disease appear on the inoculated plant.

Experiment 4. October 8, 1927, 15 seedlings were inoculated by rubbing the leaves with fragments of diseased leaves and slightly crushing them altogether between the fingers. None of these plants showed any signs of the disease.

Experiment 5. October 8, 1927, 18 seedlings were inoculated by means of needles. The inoculum was prepared in the same manner as shown in Experiment 3. Six inoculations were made on each seedling, 3 on the stem as in Experiment 3, and the other 3 on the leaves. No signs of the disease appeared on any of these plants.

Experiment 6. June 29, 1928, 49 seedlings were inoculated by needle punctures as mentioned above or by the use of capillary glass tubes containing the inoculum thrusting into the young part of the stem. All the inoculated plants remained free from the disease.

Experiment 7. July 2, 1929, 30 seedlings were inoculated by needle punctures and scratches. Five grams of the roots, stem and leaves of the diseased plant were ground in a mortar and the fluid was pressed out. A drop of the fluid with macerated tissue was placed on the stem near the ground and scratches were made with a needle through the fluid. In addition, 3 leaves of each seedling were inoculated by needle punctures in the same manner as mentioned before. None of these plants developed the slightest trace of any symptoms.

Experiment 8. July 12, 1929, 22 seedlings were inoculated by rubbing the leaves with the fragments of roots and stem of the diseased plant. None of these plants showed any signs of the disease.

Experiment 9. July 22, 1929, 15 seedlings were inoculated by needle punctures in the same manner as in Experiment 7. None of them developed any symptoms of the disease.

Experiment 10. August 8, 1930, 20 seedlings were inoculated by means of the needle puncture method devised by SEIN (1930) for the artificial transmission of the sugar cane mosaic. A slip about one inch long was cut off from a diseased leaf and placed on the leaf to be inoculated which was bent down and held tightly on a wooden label with the thumb and forefinger of the left hand. The fine insect pin

held in the right hand was thrust rapidly in and out repeatedly through different parts of the diseased leaf into the leaf to be inoculated. Four inoculations were made on each plant, one on the stem and three on the leaves, the pricking being repeated about 50 times on each point. No signs of the disease appeared on any of the inoculated plants.

As shown above, 227 rice plants were inoculated with the juice from the diseased plant by several different mechanical means. But none of these plants showed any symptoms of the disease.

It has been shown that certain virus diseases of plants are not readily transmitted by mechanical means. These are peach yellows (BLAKE, COOK and CONNORS 1921),[19] peach rosette (McCLINTOCK 1923),[128] aster yellows (KUNKEL 1926),[111] streak of sugar cane and maize (STOREY 1925),[208,209] soy bean mosaic (GARDNER and KENDRICK 1921),[69] broad bean mosaic (BÖNING 1927),[24] lily mosaic (OGILVIE and GUTERMAN 1929),[158] rosette of peanuts (STOREY and BOTTOMLEY 1928),[215] virus diseases of brambles (ZELLER 1927,[235] RANKIN 1922[168]), strawberry yellows (PLAKIDAS 1927),[164] leaf curl of cotton (KIRKPATRICK 1930,[101] 1931[102]) and others.

A number of virus diseases of plants have been reported to be transmissible by mechanical means. In some cases, however, such claims have been disproved or remain unconfirmed. For instance, SCHULTZ and FOLSOM (1923)[182] claimed that the streak of potato was communicable through the juice from the diseased plant but ATANASOFF (1922)[9] has been unable to confirm their conclusions. KASAI (1921)[98] reported that he obtained evidence of the transmission of the potato leaf roll through the juice. SCHULTZ and FOLSOM (1923)[182] and SCHAFFNIT and JÖHNSSEN (1933),[177] however, have failed to transmit it by mechanical inoculations. ROBBINS (1921)[174] and JONES (1931)[96] were unable to transmit the mosaic disease of sugar beet through the juice while BÖNING (1927),[25] working with the same disease, obtained positive results only in a few cases. TOWNSEND (1908)[221] and SMITH and BONQUET (1915)[202] failed to produce the curly top of sugar beet by artificial inoculation with the juice from the diseased plant but SEVERIN (1924)[187] claims that he succeeded in transmitting the same disease through the juice.

In the other cases various mosaic diseases of plants have been known to be transmissible through the juice of diseased plant or by leaf mutilation. These diseases are tobacco mosaic (MAYER 1886,[124] IWANOWSKI 1892[87] and others), yellow mosaic of tobacco (McKINNEY 1926,[185] JOHNSON 1927,[94] SMITH 1928[197]), ring spot of tobacco (JOHN-

SON 1925,[93] 1927,[94] FROMME, WINGARD and PRIODE 1927,[65] WINGARD 1928,[230] PRIODE 1928[166]), cucumber mosaic (DOOLITTEL 1920,[49] DOOLITTLE and WALKER 1923,[53] WALKER 1926[225]), potato mosaic (SCHULTZ, FOLSOM, HILDEBRANDT and HAWKINS 1919,[184] SCHULTZ and FOLSOM 1920,[180] 1923,[182] 1925[183]), sweet potato mosaic (ROSEN 1926),[175] sugar cane mosaic (EARLE 1919,[55] MATZ 1919,[126] BRANDES 1920[29]), wheat mosaic (MCKINNEY 1925),[133] turnip mosaic (SCHULTZ 1921,[179] GARDNER and KENDRICK 1921[70]), sweet pea mosaic (TAUBENHAUS 1914,[220] DOOLITTLE and JONES 1925[52]), bean mosaic (FAJARDO 1930),[60, 61] spinach blight (MCCLINTOCK and SMITH 1918),[129] mosaic of *Convallaria majalis* (BLATTNY 1929),[29] tulip mosaic (MCKAY, BRIERLEY, and DYKSTRA 1929,[130] ATANASOFF 1928[10]), hyacinth mosaic (ATANASOFF 1928),[10] narcissus mosaic (ATANASOFF 1928),[10] mosaic disease of *Prunella vulgaris* (LIRO 1930)[121] and others.

D) Transmission by the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL.

All evidence obtained to date indicates that dwarf disease of rice plant is transmitted only by the agency of the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL. This apparent obligate relationship between the virus and the insect suggests that the studies along this line may furnish a clue which will throw some light on the nature of the virus of the disease.

Since the symptoms of the disease are so distinct as to allow of no doubt in their recognition, dwarf disease of rice plant is fovorable for such an experimental study.

1. *On the leafhopper, Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL., *which acts as the insect vector of the disease*

Before discussing the transmission of the dwarf disease of rice plant by the agency of the leafhopper, it seems desirable to give a brief account of the morphological characters and life history of the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL.

The adult is a small yellowish green inseet with a length of 4.5 to 6 mm. and an average width of 1.6 mm. across the broadest part of the head, the male being smaller than the female. On the dorsal side: vertex yellowish green with a black transverse marking on the anterior margin; pronotum and scutellum green; elytra yellowish green, with black distal ends in the male and with those slightly tinged with gray

in the female; abdomen black. On the ventral side: thorax and abdomen light brown in the female and black in the male.

The egg is about 1 *mm.* long, yellowish white, elongate, slightly curved and tapering at one end. The eggs are deposited in the tissues of leafsheaths, usually 15 to 25 in a place and are thrust transversely into the tissues lying one under the other. At first the eggs are scarcely visible since they are pushed in so deeply that they are entirely imbedded in the tissues.

The larvae are at first creamy white and hairy with somewhat indistinct black stripes along both sides of the body. As they grow various minute black spots and an irregular pattern of dark color develop on the back of the thorax and abdomen. The dark-coloured nymphs grow into the male while the light-colored ones into the female. They are very active and leap with surprising agility.

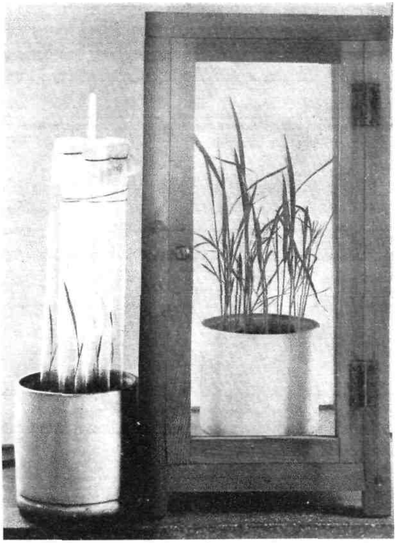
This leafhopper is a 4-, or 5-brooded insect and oviposition extends through a long period of time, which makes the seasonal history of this insect rather complicated. In general this leafhopper overwinters as a nymph, feeding on *Astragalus sinicus* L. and grasses as stated before and matures in April or May, according to the weather conditions. Later the adults begin to appear on the seed beds of rice plant. The adults commence to feed on the rice seedlings at once and if the season is sufficiently advanced, they will soon begin to lay eggs and continue to do so for a long period, each female depositing over one hundred eggs. The eggs hatch in 7–13 days and the larvae mature in about 20 days or more. The leafhoppers multiply abundantly on the rice fields after the rice seedlings are transplanted and 3 or 4 generations of the insect are produced there, the first adults of each brood developing in June to July, August, and September respectively. The eggs of the final brood of the season hatch in September to October and the nymphs hibernate among *Astragalus sinicus* L. or grasses growing on the rice fields.

2. *Viruliferous and non-viruliferous leafhoppers reared on diseased plants*

a) Results of experiments carried out in 1928*

On March 30, fifty individuals of virus-free leafhoppers, the progeny

* This series of experiments were carried out in the green house of the Tottori College of Agriculture and the work subsequent to 1930 was performed in the green house of the Faculty of Agriculture, Hokkaido Imperial University in Sapporo.



An insect cage and glass tubes employed in the writer's experiments

of leafhoppers collected the previous fall on rice plants in the vicinity of the city of Tottori where dwarf disease of rice plant has never been found to occur and retained virus free feeding on healthy rice plants in insect proof cages ,were introduced into two insect proof cages enclosing diseased rice plants in a green house. The diseased plants mentioned above were experimentally infected ones by the agency of viruliferous leafhoppers the previous fall. The offspring of the leafhoppers thus reared on diseased rice plants were fested individually for their ability to transmit the disease. Single leafhoppers were placed in glass tubes, about 30–40 cm. long by 3 cm. in diameter, closed at the upper end with a thin cotton cloth, and put on young rice seedlings 10–30 days after germination. The leafhoppers were introduced into the tubes by means of the pipette method originally used by KUNKEL (1926),[111] and allowed

to remain there for a period of 1-23 days feeding on the rice plants. Insect transfers were made before the window in a small room. Since the leafhopper has a tendency to fly towards a light source, any leafhopper that escaped when cage was opened went to the window and could be easily recaptured. The leafhoppers were often successfully arrested from escape by means of a black cloth under which the cage was opened.

TABLE 2. Results of transmission experiments with the leafhoppers reared on diseased rice plants in 1928

(Series 1. Experiments with a single leafhopper on each rice plant)

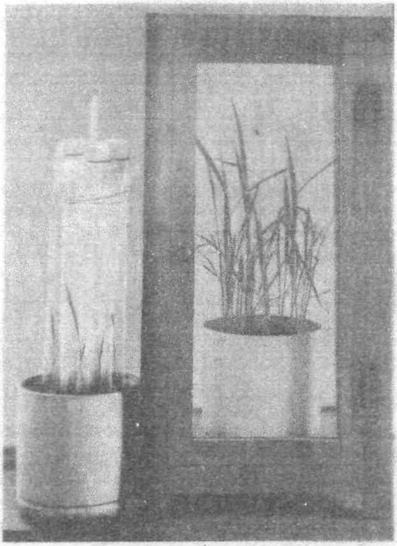
Date of begin. experiment	No. of insect tested	Sex of insect	Duration of feeding (in days)	Result
June 11	No. L 1	male	3?	—
"	2	"	"	—
"	3	"	"	—
"	4	"	8	+ (June 30)
"	5	"	"	+ (June 30)
"	6	"	"	—
"	7	"	10	+ (July 3)
"	8	"	"	—
"	9	"	"	
"	10	"	20	+ (July 1)
"	11	"	"	+ (July 9)
"	12	"	"	+ (July 9)
"	13	"	"	—
"	14	"	"	—
June 23	15	"	3	+ (July 9)
"	16	"	"	—
"	17	"	"	—
June 26	18	"	1?	—
"	19	"	"	—
"	20	"	"	—
June 27	21	"	7	+ (July 9)
"	22	"	"	—
"	23	"	"	—
July 4	24	"	3?	—
"	25	"	"	—
"	26	"	"	—
July 7	27	"	3?	—

hopp…
could …
arrested from …
was opened.

TABLE 2. Resu…

(Series 1. …

June 11			
"			
"	4		
"	5		
"	6		
"	7		19
"	8		
"	9		
"	10		
"	11		
"	12		
"	13		
"	14		
"	15		
Ju…	16		
"	17		
"	18		
June 2…	19		
"	20		
"	21		
June 27	22		
"	23		
"	24		
July 4	25		
"	26		
"			
Jul…			



An insect cage and glass tubes employed in the writer's experiments

of leafhoppers collected the previous fall on rice plants in the vicinity of the city of Tottori where dwarf disease of rice plant has never been found to occur and retained virus free feeding on healthy rice plants in insect proof cages, were introduced into two insect proof cages enclosing diseased rice plants in a green house. The diseased plants mentioned above were experimentally infected ones by the agency of viruliferous leafhoppers the previous fall. The offspring of the leafhoppers thus reared on diseased rice plants were tested individually for their ability to transmit the disease. Single leafhoppers were placed in glass tubes, about 30–40 cm. long by 3 cm. in diameter, closed at the upper end with a thin cotton cloth, and put on young rice seedlings 10–30 days after germination. The leafhoppers were introduced into the tubes by means of the pipette method originally used by KUNKEL (1926),[111] and allowed

to remain there for a period of 1–23 days feeding on the rice plants. Insect transfers were made before the window in a small room. Since the leafhopper has a tendency to fly towards a light-source, any leafhopper that escaped when cage was opened went to the window and could be easily recaptured. The leafhoppers were often successfully arrested from escape by means of a black cloth under which the cage was opened.

TABLE 2. Results of transmission experiments with the leafhoppers reared on diseased rice plants in 1928

(Series 1. Experiments with a single leafhopper on each rice plant)

Date of begin. experiment	No. of insect tested	Sex of insect	Duration of feeding (in days)	Result
June 11	No. b 1	male	3	–
,,	2	,,	,,	–
,,	3	,,	,,	–
,,	4	,,	8	+ (June 30)
,,	5	,,	,,	+ (June 30)
,,	6	,,	,,	–
,,	7	,,	10	+ (July 3)
,,	8	,,	,,	–
,,	9	,,	,,	–
,,	10	,,	20	+ (July 1)
,,	11	,,	,,	+ (July 9)
,,	12	,,	,,	+ (July 9)
,,	13	,,	,,	–
,,	14	,,	,,	–
June 23	15	,,	3	+ (July 9)
,,	16	,,	,,	–
,,	17	,,	,,	–
June 26	18	,,	1	–
,,	19	,,	,,	–
,,	20	,,	,,	–
June 27	21	,,	7	+ (July 9)
,,	22	,,	,,	–
,,	23	,,	,,	–
July 4	24	,,	5	–
,,	25	,,	,,	–
,,	26	,,	,,	–
July 7	27	,,	3	–

Date of begin. experiment	No. of insect tested	Sex of insect	Duration of feeding (in days)	Result
July 7	No. b 28	male	3	—
,,	29	,,	,,	—
,,	30	,,	,,	—
,,	31	,,	,,	—
,,	32	,,	,,	—
,,	33	,,	,,	—
July 11	34	,,	1	+ (July 23)
,,	35	,,	,,	—
,,	36	,,	,,	—
,,	37	,,	,,	—
,,	38	,,	,,	—
,,	39	,,	,,	+ (July 30)
,,	40	,,	,,	—
,,	41	,,	,,	—
,,	42	,,	,,	—
,,	43	,,	,,	—
Aug. 7	44	,,	,,	—
,,	45	,,	,,	—
,,	46	,,	,,	—
,,	47	,,	,,	—
,,	48	,,	,,	—
,,	49	,,	,,	—
Aug. 28	50	,,	7	—
,,	51	,,	23	—
,,	52	,,	11	—
,,	53	,,	23	—
,,	54	,,	,,	—
,,	55	,,	13	—
,,	56	,,	23	—
,,	57	,,	,,	—
,,	58	,,	,,	—
,,	59	,,	,,	—
,,	60	,,	,,	—
,,	61	,,	,,	—
,,	62	,,	10	—
,,	63	,,	23	—
,,	64	,,	,,	—
,,	65	,,	10	—

Date of begin. experiment	No. of insect tested	Sex of *insect*	*Duration of feeding* (in days)	*Result*
Aug. 28	No. b 66	male	23	—
,,	67	,,	,,	—
,,	68	famale	,,	—
,,	69	,,	11	—
,,	70	,,	7	—
,,	71	,,	13	—
,,	72	,,	23	—
,,	73	,,	7	—
,,	74	,,	23	—
,,	75	,,	,,	—
,,	76	,,	10	+ (Oct. 1)
,,	77	,,	23	—
,,	78	,,	11	—
,,	79	,,	23	—
,,	80	,,	,,	—
,,	81	,,	,,	—
,,	82	,,	,,	—
,,	83	,,	,,	—
,,	84	,,	,,	—
,,	85	,,	,,	—
,,	86	,,	,,	—
,,	87	,,	,,	—

(Series 2. Experiments with more than one leafhopper on each plant)

Date of begin. experiment	No. of insects per plant	Duration of feeding (in days)	Result
June 26	3 (male)	1	+ (July 10)
,,	,,	,,	+ (July 11)
,,	,,	,,	—
June 23	,,	3	+ (July 3)
,,	,,	,,	+ (July 7)
,,	,,	,,	—
June 27	,,	7	+ (July 12)
,,	,,	,,	+ (July 12)
,,	,,	,,	—
May 31	,,	10	+ (June 21)
,,	,,	,,	—

Date of begin. experiment	No. of insects per plant	Duration of feeding (in days)	Result
May 31	3 (male)	10	−
,,	,,	,,	−
,,	,,	,,	−
,,	,, (female)	,,	−
,,	,, (nymph)	,,	+ (July 6)
,,	,,	,,	−
,,	,,	,,	−
,,	,,	,,	−
,,	,,	,,	−
,,	,,	,,	−
June 26	5 (male)	1	−
,,	,, (female)	,,	−
,,	,,	,,	+ (July 12)
June 23	5 (male)	3	−
,,	,, (female)	,,	−
,,	,,	,,	+ (July 9)
June 27	5 (male)	7	+ (July 9)
June 9	,, (nymph)	10	+ (July 6)
,,	,,	,,	−
,,	,,	,,	−
,,	,,	12	−
,,	,,	,,	−
,,	,,	,,	−
June 26	10 (female)	1	−
,,	,,	,,	−
June 23	,, (male)	3	+ (July 9)
,,	,, (female)	,,	−
,,	,,	,,	−
June 27	,, (male)	7	+ (July 16)

b) Results of experiments carried out in 1930–1931

On April 14, eighty or ninety leafhoppers were collected on *Astragalus sinicus* L. growing on the rice fields in the vicinity of Utsunomiya and two days later about half of these insects were placed in insect proof cages containing dwarf diseased rice plants in the green house. Some of these insects were females, and each produced a numerous progeny. The first nymphs hatched on May 5 and the first adult was bred on June 7 requiring about one month to complete the nymphal

instars. The adults of the second, third and fourth generation began to appear on August 25, December 10 and March 10, 1931, respectively. The adult leafhoppers thus reared on diseased rice plants were tested individually for their capacity to transmit the disease in the same way as shown before. The results of the experiments will be shown in the following table.

TABLE 3. Results of the transmission experiments with the leafhoppers reared on diseased rice plants in 1930–1931.

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
June 18	No. c 1	male	68	18–34°C	–
,,	2	,,	,,	,,	–
,,	3	,,	13	14–30	–
July 2	4	,,	19	15–33	+ (July 20)
Aug. 2	5	,,	21	21–36	–
,,	6	,,	,,	,,	–
,,	7	,,	,,	,,	–
July 11	8	,,	45	20–36	–
,,	9	,,	28	19–36	+ (Aug. 8)
,,	10	,,	45	20–36	–
,,	11	,,	,,	,,	–
,,	12	,,	25	19–36	–
,,	13	,,	,,	,,	–
,,	14	,,	,,	,,	–
,,	15	female	,,	,,	–
July 21	16	,,	14	21–36	–
,,	17	,,	45	20–34	+ (Aug. 14)
Aug. 11	18	male	55	17–35	–
,,	19	,,	,,	,,	–
,,	20	,,	,,	,,	–
,,	21	,,	86	15–35	–
,,	22	,,	55	19–35	–
Sept. 1	23	,,	54	14–36	–
,,	24	,,	30	15–36	+ (Oct. 7)
,,	25	,,	17	16–36	–
,,	26	,,	,,	,,	+ (Sept. 18)
,,	27	,,	16	,,	+ (Sept. 17)
Sept. 15	28	,,	25	14–36	+ (Oct. 10)

Date of begin. experiment	No. of insect tested	Sex	Duration of feediug (in days)	Temperature	Result
Sept. 15	No. c 29	male	52	14–35°C	–
,,	30	,,	22	15–36	+ (Oct. 7)
,,	31	,,	35	14–36	–
,,	32	,,	23	,,	+ (Oct. 8)
,,	33	,,	35	,,	–
,,	34	,,	22	15–36	–
,,	35	,,	30	14–36	+ (Oct. 15)
,,	36	,,	25	,,	+ (Oct. 10)
Dec. 27	37	,,	37	17–31	–
,,	38	,,	,,	,,	–
,,	39	,,	32	,,	–
,,	40	,,	,,	,,	–
,,	41	,,	37	,,	–
,,	42	,,	48	,,	–
,,	43	,,	25	,,	–
Jan. 7	44	,,	26	,,	–
,,	45	,,	,,	,,	–
,,	46	,,	34	,,	–
,,	47	,,	26	,,	–
,,	48	,,	34	,,	–
,,	49	,,	26	,,	–
,,	50	,,	,,	,,	–
,,	51	,,	34	,,	–
,,	52	,,	66	18–34	–
,,	53	,,	26	17–31	–
,,	54	,,	34	,,	–
,,	55	,,	44	,,	–
,,	56	,,	,,	,,	–
,,	57	,,	,,	,,	–
,,	58	,,	34	,,	–
,,	59	,,	44	,,	–
Mar. 24	60	,,	,,	19–34	–
,,	61	,,	27	18–33	–
,,	62	,,	7	18–35	–
,,	63	female	46	19–34	–
,,	64	,,	57	19–35	–
Mar. 30	65	male	21	18–33	–
,,	66	female	26	,,	–

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
Mar. 30	No. c 67	female	26	18–33°C	–
Apr. 4	68	male	29	19–33	–
,,	69	,,	24	,,	–
Apr. 15	70	,,	10	20–35	–
,,	71	,,	35	20–36	–
,,	72	,,	8	19–35	–
Apr. 25	73	,,	34	19–34	–
,,	74	,,	,,	,,	–
,,	75	,,	25	21–36	–

c) Results of experiments carried out in 1931–1932

On August 14, about 30 non-viruliferous leafhoppers, the progeny of the insects maintained virus free since they had been collected on *Astragalus sinicus* L. the previous year, were placed in an insect proof cage containing dwarf diseased rice plants. The offspring of these leafhoppers reared on diseased rice plants were tested individually for their ability to transmit the disease. The results of the experiments will be shown in the following table. Since almost all the leafhoppers failed to produce infections in healthy plants, the experimental results will be presented in an abridged manner.

TABLE 4. Results of the transmission experiments with the leafhoppers reared on diseased rice plants in 1931–1932

Date of begin. experiment	No. of insects tested	Sex	Duration of feeding (in days)	Temperature	No. of viruliferous insects
Nov. 28	7	male	11–20	19–30°C	0
,,	2	,,	31–40	20–31	0
,,	1	,,	51–60	19–30	0
,,	1	female	1–10	23–25	0
,,	3	,,	11–20	19–30	0
,,	3	,,	21–30	20–31	0
,,	2	,,	31–40	,,	0
,,	1	,,	51–60	19–30	0
Dec, 1	6	male	11–20	20–31	0
,,	5	,,	21–30	21–32	0

Date of begin. experiment	No. of insects tested	Sex	Duration of feeding (in days)	Temperature	No. of viruliferous insects
Dec. 1	4	male	31–40	20–31°C	0
,,	2	,,	41–50	19–31	1 (Jan. 11)
Dec. 7	1	,,	1–10	21–31	0
,,	3	,,	11–20	,,	0
,,	1	,,	21–30	,,	0
,,	5	,,	31–40	20–31	0
Dec. 13	3	,,	1–10	21–32	0
,,	20	,,	11–20	,,	0
,,	2	,,	21–30	20–32	0
,,	1	,,	31–40	19–30	0
,,	6	,,	41–50	,,	0
,,	4	,,	51–60	19–31	0
,,	2	,,	71–80	,,	0
Dec. 16	1	,,	1–10	20–31	0
,,	4	,,	11–20	,,	0
,,	11	,,	21–30	19–31	0
,,	5	,,	31–40	19–30	0
,,	3	,,	41–50	19–31	0
Dec. 26	1	,,	1–10	20–31	0
,,	1	,,	11–20	19–29	0
,,	1	,,	21–30	19–30	0
,,	1	,,	31–40	,,	0
,,	3	,,	41–50	,,	0
,,	3	,,	51–60	,,	0
,,	1	nymph	21–30	,,	0
,,	1	,,	41–50	,,	0
,,	4	,,	51–60	,,	0
,,	6	,,	61–70	18–30	0
,,	7	,,	81–90	19–31	0
Jan. 26	2	male	1–10	18–31	0
,,	7	,,	11–20	19–31	0
,,	7	,,	21–30	18–31	0
,,	4	,,	31–40	,,	0
,,	6	,,	41–50	,,	0
,,	1	,,	51–60	18–32	0

d) Results of experiments carried out in 1932–1933

On April 13, about 200 leafhoppers were collected on *Astragalus sinicus* L. growing on the rice fields in the vicinity of Shiraoka near Ômiya and two days later about half their number were introduced into insect proof cages enclosing dwarf diseased rice plants in the green house. The progeny of these insects thus reared on diseased rice plants were tested individually for their ability to transmit the disease in the same way as stated before: viz., each rice seedling was exposed to a single leafhopper in a glass tube throughout its life. The results of the experiments will be shown in the following table.

TABLE 5. *Results of transmission experiments with the leafhoppers* reared on diseased rice plants in 1932–1933

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
June 14	No. c 1	male	42	18–33°C	—
,,	2	,,	40	,,	—
,,	3	,,	,,	,,	—
,,	4	,,	33	17–33	—
,,	5	,,	38	,,	—
,,	6	,,	20	16–33	—
,,	7	,,	40	18–33	—
,,	8	,,	33	17–33	—
,,	9	,,	40	18–33	—
,,	10	,,	35	17–33	—
,,	11	,,	60	18–34	—
June 20	12	,,	54	19–34	—
,,	13	,,	,,	,,	—
,,	14	,,	,,	,,	—
,,	15	,,	62	18–34	—
,,	16	,,	37	18–33	—
,,	17	,,	,,	,,	—
,,	18	,,	18	17–33	—
,,	19	,,	28	,,	—
,,	20	,,	20	,,	—
,,	21	,,	17	,,	—
,,	22	,,	12	,,	—

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
June 20	No. e 23	male	40	18–33°C	−
”	24	”	54	19–34	−
”	25	”	36	18–33	−
”	26	”	40	”	−
”	27	”	33	”	−
”	28	”	25	17–32	−
”	29	”	32	18–33	−
”	30	”	”	”	−
”	31	”	33	”	−
June 24	32	”	30	”	−
”	33	”	24	17–33	−
”	34	”	33	18–33	−
”	35	”	25	”	+ (Aug. 3)
”	36	”	30	”	−
”	37	”	44	”	−
”	38	”	24	17–33	−
”	39	”	30	18–33	−
”	40	”	32	”	−
”	41	”	47	18–34	−
”	42	”	39	18–33	−
”	43	”	8	16–33	−
”	44	”	24	17–33	−
”	45	”	58	18–34	−
”	46	”	33	18–33	−
”	47	”	9	16–33	−
”	48	”	14	17–33	−
July 7	49	”	12	18–32	−
”	50	”	11	”	−
”	51	”	7*	16–31	−
”	52	”	12	18–32	+ (July 20)
”	53	”	”	”	−
”	54	”	11	”	+ (July 20)
”	55	”	12	”	−
”	56	”	”	”	+ (July 20)
”	57	”	13	”	−
”	58	”	11	”	−
”	59	”	”	”	−
”	60	”	12	”	−

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
July 7	No. e 61	female	12	18-32°C	—
"	62	"	"	"	—
"	63	"	11	"	+ (July 24)
"	64	"	"	"	—
"	65	"	12	"	—
"	66	"	"	"	—
"	67	"	20	18-34	—
"	68	"	12	18-32	—
"	69	"	"	"	—
"	70	"	11	"	—
"	71	male	12	"	—
"	72	"	11	"	—
"	73	"	7	16-31	—
"	74	"	12	18-32	—
"	75	"	"	"	—
"	76	"	"	"	—
"	77	"	"	"	—
"	78	"	"	"	—
"	79	"	"	"	+ (July 20)
"	80	"	"	"	—
"	81	"	"	"	+ (July 21)
July 20	82	"	20	20-36	—
"	83	"	"	"	—
"	84	"	21	"	—
"	85	"	"	"	—
"	86	"	18	"	—
"	87	"	"	"	—
"	88	"	24	"	—
"	89	"	5	20-37	—
"	90	"	14	20-36	—
"	91	"	"	"	—
"	92	"	"	"	—
"	93	"	"	"	—
"	94	"	7	21-36	—
"	95	"	20	20-36	—
"	96	"	24	"	—
"	97	"	"	"	—
"	98	"	28	"	—

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
July 20	No. c 99	female	11	21–36°C	–
,,	100	,,	26	20–36	–
,,	101	,,	16	,,	+ (Aug. 5)
,,	102	,,	,,	,,	–
,,	103	,,	11	21–36	+ (Aug. 5)
,,	104	,,	62	18–34	–
,,	105	,,	24	20–36	–
,,	106	,,	16	,,	+ (Aug. 5)
,,	107	,,	32	,,	–
,,	108	,,	11	21–36	+ (Aug. 5)
,,	109	,,	24	20–36	–
July 30	110	male	5	18–36	–
,,	111	,,	11	,,	–
,,	112	,,	22	19–36	–
,,	113	,,	,,	,,	–
,,	114	,,	16	20–36	+ (Aug. 15)
,,	115	,,	17	,,	–
,,	116	,,	37	19–36	–
,,	117	,,	5	18–36	–
,,	118	,,	11	,,	–
,,	119	,,	14	20–36	–
,,	120	,,	21	,,	–
,,	121	,,	43	18–35	–
,,	122	,,	10	18–36	–
,,	123	,,	11	,,	–
,,	124	,,	22	19–36	–
,,	125	,,	38	,,	–
,,	126	,,	18	20–36	–
,,	127	female	17	,,	–
,,	128	,,	14	,,	+ (Aug. 24)
,,	129	,,	17	,,	+ (Aug. 19)
,,	130	,,	5	18–36	–
,,	131	,,	14	20–36	+ (Aug. 18)
,,	132	,,	17	,,	–
Aug. 15	133	male	31	18–34	–
,,	134	,,	21	18–35	–
,,	135	,,	27	18–34	–
,,	136	,,	10	18–36	–

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
Aug. 15	No. c 137	male	12	18–36°C	—
,,	138	,,	31	18–34	—
,,	139	,,	4	17–37	+ (Aug. 23)
,,	140	,,	16	18–36	—
,,	141	,,	8	18–37	—
,,	142	,,	22	18–35	—
,,	143	,,	31	18–34	—
,,	144	,,	9	18–37	+ (Aug. 24)
,,	145	,,	6	18–36	—
,,	146	,,	,,	,,	—
,,	147	female	,,	,,	+ (Aug. 26)
,,	148	,,	,,	,,	—
,,	149	,,	,,	,,	—
,,	150	,,	,,	,,	—
,,	151	,,	14	18–37	+ (Sept. 15)
,,	152	,,	10	18–36	—
,,	153	,,	,,	,,	—
,,	154	,,	,,	,,	—
,,	155	,,	9	18–37	—
,,	156	,,	,,	,,	—
,,	157	,,	22	18–35	—
,,	158	,,	9	18–37	—
,,	159	,,	15	,,	—
,,	160	,,	,,	,,	—
,,	161	,,	,,	,,	—
,,	162	,,	10	18–36	—
Sept. 6	163	male	39	13–33	—
,,	164	,,	54	14–33	—
,,	165	,,	,,	,,	—
,,	166	,,	46	,,	—
,,	167	,,	37	13–33	—
,,	168	,,	23	13–32	—
,,	169	,,	,,	,,	+ (Sept. 29)
,,	170	,,	10	16–34	—
,,	171	,,	,,	,,	—
,,	172	,,	43	13–33	—
,,	173	,,	,,	,,	—
,,	174	,,	52	14–33	—

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
Sept. 6	No. e 175	male	39	13-33°C	—
,,	176	,,	23	13-32	—
,,	177	,,	39	13-33	—
,,	178	,,	54	14-33	—
,,	179	,,	59	15-34	—
,,	180	,,	54	14-33	—
,,	181	,,	10	16-34	+ (Sept. 29)
,,	182	,,	52	14-33	—
,,	183	,,	59	15-34	—
,,	184	,,	42	13-33	—
,,	185	,,	23	13-32	—
,,	186	,,	33	13-33	—
,,	187	,,	42	,,	—
,,	188	,,	23	13-32	—
,,	189	,,	42	13-33	—
,,	190	,,	23	13-32	—
,,	191	female	,,	,,	—
,,	192	,,	42	13-33	—
,,	193	,,	,,	,,	—
,,	194	,,	,,	,,	—
,,	195	,,	26	13-32	—
,,	196	,,	33	13-33	—
Sept. 7	197	male	9	16-33	—
,,	198	,,	36	12-33	—
,,	199	,,	,,	,,	+ (Oct. 22)
,,	200	,,	40	13-33	—
,,	201	,,	9	16-33	—
,,	202	,,	34	13-33	—
,,	203	,,	22	,,	—
,,	204	,,	,,	,,	—
,,	205	,,	,,	,,	—
,,	206	,,	41	,,	—
,,	207	,,	22	,,	+ (Sept. 29)
,,	208	,,	,,	,,	—
,,	209	female	,,	,,	—
,,	210	,,	45	14-33	—
,,	211	,,	58	15-34	—
,,	212	,,	22	13-33	—

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
Sept. 7	No. e 213	female	22	13–33°C	–
„	214	„	25	„	–
„	215	„	41	„	–
„	216	„	25	„	–
„	217	„	22	„	+ (Sept. 29)
„	218	„	„	„	–
„	219	„	„	„	–
„	220	„	9	16–33	–
„	221	„	41	13–33	–
„	222	„	22	„	–
„	223	„	59	15–34	–
„	224	„	22	13–33	–
„	225	„	59	15–34	–
„	226	„	32	13–33	–
„	227	„	45	14–33	–
Sept. 8	228	male	6	16–33	+ (Sept. 25)
„	229	„	27	14–32	–
„	230	female	31	13–33	+ (Oct. 19)
„	231	„	8	16–33	+ (Sept. 29)
„	232	„	40	13–33	–
„	233	„	21	„	–
„	234	„	8	16–33	–
„	235	„	40	13–33	–
Sept. 22	236	male	10	13–31	+ (Oct. 2)
„	237	„	44	14–34	–
„	238	„	„	„	–
„	239	„	„	„	–
„	240	„	7	13–32	+ (Sept. 29)
„	241	„	39	14–34	–
„	242	„	32	13–34	–
„	243	„	44	14–34	–
„	244	„	38	„	–
„	245	„	44	„	–
„	246	„	39	„	–
„	247	„	12	13–33	–
„	248	„	44	14–34	–
„	249	„	„	„	–
„	250	female	10	13–31	–

Date of begin. experiment	No. of insect tested	Sex	Duration of feeding (in days)	Temperature	Result
Sept. 22	No. c 251	female	26	12–33°C	—
,,	252	,,	,,	,,	—
,,	253	,,	10	13–31	—
,,	254	,,	44	14–34	—
,,	255	,,	27	13–33	—
,,	256	,,	13	13–32	+ (Oct. 5)
,,	257	,,	44	14–34	—
Sept. 30	258	male	22	,,	—
,,	259	,,	11	12–33	—
,,	260	,,	22	14–34	—
,,	261	,,	,,	,,	—
,,	262	,,	2	15–31	—
,,	263	female	22	14–34	—
,,	264	,,	,,	,,	—
,,	265	,,	31	17–34	—
,,	266	,,	36	,,	—
,,	267	,,	22	14–34	—
,,	268	,,	,,	,,	—
,,	269	,,	19	12–34	—
,,	270	,,	36	17–34	—
,,	271	,,	,,	,,	—
,,	272	,,	11	12–33	+ (Oct. 11)
,,	273	,,	36	17–34	—
,,	274	,,	8	12–34	—
Oct. 19	275	,,	12	19–35	+ (Oct. 31)
,,	276	,,	17	20–34	—
,,	277	,,	,,	,,	—
,,	278	,,	11	19–34	—
,,	279	,,	7	,,	+ (Oct. 26)
,,	280	,,	17	20–34	—
,,	281	,,	15	,,	—
,,	282	,,	3	,,	—

Subsequent to November 15, almost all leafhoppers tested failed to produce infection in healthy plants and the results will be presented in an abridged manner.

Date of begin. experiment	No. of insects tested	Sex	Duration of feeding (in days)	Temperature	No. of viruliferous insects
Nov. 15	1	male	21–30	20–30°C	0
"	1	"	31–40	"	0
"	1	female	1–10	19–30	0
"	3	"	11–20	"	0
Dec. 1	1	male	1–10	20–31	0
"	1	"	11–20	"	0
"	2	female	"	"	0
Mar. 3	6	male	20	20–33	0
4	4	"	"	"	0
"	1	female	"	"	0
6	14	nymph	"	"	0
15	18	male	"	19–35	0
17	5	"	"	"	0
"	1	female	"	"	0
19	6	male	"	19–36	0
Apr. 24	5	"	"	20–38	0
May 12	7	"	"	20–35	0
June 1	8	"	"	13–35	0
22	10	"	"	18–38	0

The results shown in the above tables will be summarized as follows.

TABLE 6. Summarized results of transmission experiments with the leafhoppers reared on diseased rice plants

Year	Duration of feeding (in days)	No. of insects tested	No. of infective insects	Percent. of infective insects
1928	1–10	50 (♀3)	8 (♀1)	16
	11–20	10 (♀3)	3	30
	21–30	27 (♀14)	0	0
		87	11	13
1930	1–10	3	0	0
	11–20	6 (♀1)	4 (♀1)	67
	21–30	69 (♀3)	7	24
	31–40	15	0	0
	41–50	11 (♀2)	0	0
	51–60	7 (♀1)	0	0
	61–70	3	0	0
	81–90	1	0	0
		75	11	15

Year	Duration of feeding (*in days*)	No. of insects tested	No. of infective insects	Percent. of infective insects
1931	1–10	9 (♀1)	0	0
	11–20	52 (♀3)	0	0
	21–30	31 (♀3, n* 1)	0	0
	31–40	22 (♀2)	0	0
	41–50	20 (n 1)	1	5
	51–60	14 (♀1, n 4)	0	0
	61–70	6 (n 6)	0	0
	71–80	2	0	0
	81–90	7 (n 7)	0	0
		163	1	0.6
1932	1–10	46 (♀24)	9 (♀3)	20
	11–20	173 (♀52, n 14)	19 (♀15)	11
	21–30	61 (♀24)	4 (♀1)	7
	31–40	51 (♀12)	2 (♀1)	4
	41–50	27 (♀9)	0	0
	51–60	17 (♀4)	0	0
	61–60	2 (♀1)	0	0
		377	34	9

* n stands for nymph.

As shown above, 702 leafhoppers which had been reared upon dwarf diseased rice plants were tested singly for their capability of transmitting the disease. and only 57 (or 8 per cent) of them proved to be infective. Of the leafhoppers tested 506 were male, 163 female and the others nymphs. Among them 35 males and 22 females proved to be infective, the percentage of infective males and females amounting to 7 and 13, respectively. These infective leafhoppers were able to produce infections in healthy rice plants when allowed to feed upon them for a comparatively short period. The results shown above appear to justify the conclusion that certain individuals of the leafhoppers reared on dwarf-diseased rice plants are incapable of tarnsmitting the disease when single leafhoppers are confined to each healthy rice plant.

In his studies on aster yellows, KUNKEL (1926)[111] found that "most, if not all, individuals of *Cicadula sexnotata* FALL. were capable of taking up the aster yellow virus" when they were allowed to feed on yellow diseased aster plants. STOREY (1925,[210] 1928[212]), on the other

hand, asserted that certain individuals of the leafhopper, *Cicadulina mbila* (*Balclutha mbila* NAUDE) remained uninfective, even when they fed upon streak diseased maize plant through the whole course of development of the insect from the first instar to the adult stage.

3. *Length of time infective leafhoppers must remain on rice plants to transmit the disease*

A series of experiments were carried out to ascertain the minimum length of time infective leafhoppers must remain on healthy rice plants to transmit the disease. Single leafhoppers, experimentally proved to

TABLE 7. Results of experiments on the relation between the length of feeding period and the number of infection obtained

Date	Insect No.	Sex	Length of time insects remained on plants				
			5	10	20	30	60 (min.)
Sept. 22'30	c 26	♂					—
Sept. 10'30	c 30	,,					—
,,	c 32	,,					+ (21)
Aug. 19'32	e 114	,,				+ (18)	+ (19)
Oct. 4'32	e 169	,,	—	—	—	+ (13)	+ (25)
,,	e 207	,,	—	—	—	—	—
,,	e 236	,,	—	—	+ (16)	+ (21)	+ (14)
,,	e 240	,,	—	—	—	—	—
Oct. 5'32	e 256	♀	—	—	—	+ (25)	+ (18)
Oct. 12'32	c 272	,,	—	—	+ (25)	+ (15)	+ (12)
Oct. 25'32	e 151-4	,,	—	—	—	+ (12)	+ (10)
Oct. 27'32	e 151-1	,,	—	+ (9)	—	+ (9)	+ (9)
Jan. 2'33	e 151-4-13	,,	—	—	—	—	+ (10)
Jan. 7'33	e 151-1-26	,,	+ (19)	—	+ (19)	—	—
,,	e 151-1-6	,,	+ (24)	+ (12)	+ (17)	+ (16)	+ (16)
Jan. 9'33	e 151-1-13	♂	—	—	—	+ (14)	+ (14)
			2/12	2/12	4/12	9/13	11/16
Length of incubation period (in days)			10–24	9–12	16–25	9–25	9–25

The numbers in parentheses indicate incubation periods in days.

be infective, were confined to each young rice plant in a glass tube. When the leafhopper alighted on the leaf of rice plant and appeared to begin sucking the juice, the time was recorded and the insect was allowed to remain there during a definite length of time. It was not definitely known whether the leafhopper was actually sucking the juice all the time. The results of the experiments will be shown in table 7.

As shown in the table, most of the viruliferous leafhoppers were capable of transmitting the disease to healthy plants when they were allowed to feed on the plants for 30 to 60 minutes, and as short a period as 5 minutes was sufficient to transmit the disease. This indicates that only a small amount of virus may produce a typical case of dwarf disease of rice plant. It presents a striking contrast that certain individuals of the leafhoppers reared on dwarf diseased rice plants are capable of transmitting the disease to healthy plants after only 30–60 minutes' feeding whereas some leafhoppers from the same culture on diseased plants cannot produce infections in healthy plants even though allowed to feed on them as long as they live. SMITH and BONCQUET (1915),[202] working with the curly top of beets, reported that exposure of beets for 5 minutes to feeding by infective leafhoppers was sufficient to cause the disease. In their studies on spinach blight, McCLINTOCK and SMITH (1918)[129] also found that virus bearing aphids (*Macrosiphum solanifolii* and *Rhopalosiphum persicae*) produced infections in healthy plants when allowed to feed on them for 5 minutes. It seems that these investigators worked with a lot of insects, instead of single aphids. According to KURIBAYASHI (1931),[116] stripe disease of rice plant was transmitted to healthy plants when single infective leafhoppers (*Delphacodes striatellus* FALL.) were allowed to feed on them for a period of 5 hours.

Incubation periods varied, as shown in the table, from 9 to 25 days, nearly as long as those when the feeding time was prolonged to one day, the incubation periods in the latter case ranging from 6 to 37 days, generally from 8 to 24 days.

4. *Retention of infective power by the leafhopper*

In 1915 Murata stated in his manual entitled "Insect pests of rice, barley and wheat and their control measures", that infective leafhoppers would partly or entirely lose their ability to induce the dwarf disease of rice plant when they were repeatedly transferred to new healthy plants at 2 or 3 day intervals. He has published, however, no papers presenting his experimental data. Since this aspect of the

problem seems to have significant bearing upon the nature of the virus, it has been further investigated by the present writer by transferring single infective leafhoppers daily or at several day intervals to new healthy rice plants. Single leafhoppers, experimentally proved to be infective were confined to each young rice plant of "Aikoku"-variety, 10 to 30 days after germination, in a glass tube, allowed to remain there during a definite length of time and then transferred to new healthy plants. The results of experiments will be presented in the tables, 8 and 9.

TABLE 9. Results of transmission experiments with infective leafhoppers transferring the insects to new healthy plants at several day intervals (1930 and 1932)

e 4 (♂)	July	21–22	22–23	23–30	30–31	31–2	2–5	5–8	8–9	9–11	11–14	14–21.
		+	+	+	+	+	+	+	+	+	+	+
e 30 (♂)	Oct.	10–13	13–16	16–23	23–27	27–30	30–2	2–5	5–8	8–11.		
		−	−	−	+	−	−	−	−	+		
e 32 (♂)	Oct.	10–13	13–16	16–20	20–23	23–27	27–30	30–2	2–5	5–8	8–22.	
		+	+	+	+	+	+	+	+	−	+	
e 114 (♂)	Aug.	16–19	19–22	22–25	25–28	28–31	31–3	3–6	6–9.			
		+	+	+	+	+	+	+	−			
e 169 (♂)	Sept.	29–2	2–5	5–8	8–11	11–13.						
		+	+	+	+	+						
e 236 (♂)	Oct.	2–5	5–8	8–11	11–14	14–17.						
		+	+	+	+	+						
e 240 (♂)	Sept.	29–2	2–5	5–8	8–11	11–14	14–17	17–20.				
		+	+	+	+	+	+	+				

As shown in these tables, there is a marked variability as to the infectivity of each viruliferous individual of leafhoppers. Most leafhoppers retained their infective power as long as they lived or during a period varying from 12 to 33 days, and certain leafhoppers produced infections in every healthy plant on which it was confined for 24 hours on consecutive days. In comparatively few leafhoppers, however, their infective power was apparently reduced or lost even under favorable conditions, when each individual was supplied with a healthy rice plant at one to several day intervals. The leafhopper, e279–1 which lived for a period over 50 days retained the virus for 33 days and then remained free from virus throughout its subsequent life. BONCQUET and STAHL

(1917)[23] reported that the ability of the beet leafhopper, *Eutettix tenella* BAKER, to transmit curly top was lost in from 15 to 35 days if the insects were transferred daily to healthy beets. SEVERIN (1924), on the other hand, stated that infective beet leafhoppers retained their infectivity during all of the nymphal stages, after each molt, and during the entire adult life. CARSNER (1919)[33] kept infective beet leafhoppers on apparently non-susceptible plants for over one hundred days without loss of infectivity. In his studies on aster yellows, KUNKEL (1926)[111] found that while many individuals of the leafhopper, *Cicadula sexnotata* FALL. retained the virus through life, some individuals seemed to lose it after a short time. In the case of *Cicadulina (Balclutha) mbila* NAUDE which acts as the vector of maize streak disease, STOREY (1928)[212] reports that "while the infective power is usually not lost by the leafhopper, such loss may occasionally occur."

Incubation periods varied from 6 to 37 days, generally 8 to 24 days, when single leafhoppers were confined on each seedling plant for one day. It seems that the length of incubation period depends not only upon the infective power of the leafhopper and the duration of its feeding period but also upon the growing state of the infected plants as well as the environmental conditions which affect plant growth. Especially, low temperatures retard the development of the disease.

It is worthy of note that not all the infested plants contracted the dwarf disease. Occasionally plants in the progressive series of infections remained healthy whereas the other plants in the same series developed the disease, as shown in the cases of leafhoppers, e256, e151–4–9, e279–1 etc. Similar cases have been recorded in the curly top of sugar beet and other virus diseases of plants. In their studies on the curly top of beets, CARSNER and STAHL (1924)[34] state that "a viruliferous leafhopper may not produce the disease each time it feeds on a healthy plant, even though the periods of feeding be 24 or 48 hours or even longer." STOREY (1928)[212] in his studies of maize streak disease finds that when viruliferous single leafhoppers were transferred daily to new healthy plants, frequent failures of infection occurred on some plants upon which they fed. SAMUEL et al. (1930)[176] working with "spotted wilt" of tomatoes and its transmission by the thrips, *Frankliniella insularis*, find that infective individuals fed for successive days on healthy tomato plants did not infect every plant on which they fed. SMITH (1931)[201] in his studies on the insect transmission of potato leafroll writes that occasionally plants in the pro-

gressive series of infections with viruliferous aphids failed to develop leafroll, although plants before and after in the series became infected.

Under the writer's experimental conditions the failures of infection on plants, upon which viruliferous leafhoppers fed, may be partly explained on the assumption that the leafhoppers had not actually fed on those plants, because some leafhoppers could survive 3 or 4 days without feeding, as shown by the following experiment.

Each leafhopper was kept in a glass tube as mentioned before, containing no food plant and standing on the moistened soil in a pot. The number of insects dead was recorded daily until they all died out. The results are shown in the following table.

TABLE 10. Results of starvation experiments with the leafhopper

Series 1. With insects reared on dwarf diseased rice plants (Jan. 22–Mar. 9 1933, temp: 18–32°C)

Duration of starvation (in hours)	No. of insects dead	No. of insects survived
18–24	9 (♀ 9, ♂ 0)	21 (♀ 18 ♂ 3)
24–48	6 (♀ 5, ♂ 1)	15 (♀ 13 ♂ 2)
48–72	12 (♀ 10, ♂ 2)	3 (♀ 3 ♂ 0)
72–96	1 (♀ 1, ♂ 0)	2 (♀ 2 ♂ 0)
96–120	2 (♀ 2, ♂ 0)	0
	30 (♀ 27, ♂ 3)	

Series 2. With insects reared on healthy rice plants (*Jan. 5–14 1933, temp: 20–28°C*)

Duration of starvation (in hours)	No. of insects dead	No. of insects survived
18–24	12 (♀ 8, ♂ 4)	18 (♀ 11 ♂ 7)
24–48	5 (♀ 3, ♂ 2)	13 (♀ 8 ♂ 5)
48–72	8 (♀ 6, ♂ 2)	5 (♀ 2 ♂ 3)
72–96	4 (♀ 1, ♂ 3)	1 (♀ 1 ♂ 0)
96–120	1 (♀ 1, ♂ 0)	0
	30 (♀ 19, ♂ 11)	

Only a small portion of the leafhoppers could survive more than 3 days without food as shown in the table. And moreover it can be hardly explained why the leafhoppers would not take food for as long

as 5 days, as in the cases of e151-4-9 and e151-1-23 in table 7, in spite of the presence of food plants. As a matter of fact, no leafhopper was observed remaining apart from the food plant longer than a day. At least in such cases, one inevitably supposes that certain viruliferous leafhoppers occasionally fail to produce infections in healthy plants, probably owing to the temporary exhaustion of the virus in the salivary glands and the anterior portion of the alimentary tract.

As will be stated later, the virus of dwarf disease of rice plant may be transmitted through eggs from viruliferous female leafhoppers to their offspring and certain nymphs emerging from these viruliferous eggs are capable of producing infections in healthy rice plants. Accordingly it seemed desirable to determine how long the virus is retained in the insect body when these viruliferous nymphs are transferred daily to new healthy rice plants. Single nymphs which had emerged from the eggs of viruliferous females were confined on each young rice plant of "Bôzu No. 5" variety in a glass tube, allowed to remain there one day and transferred to new healthy plants. The tiny nymphs which had just emerged from eggs could be readily transferred to rice plants by means of a small camel's hair brush as will be described later. However, some difficulties were experienced to manipulate the nymphs 2-5 days of age, since they are very active and leap with surprising agility. The transfer could be best effected by means of the pipette method as stated before, putting the nymph on the soil near the rice plant on to which it later crawled up. When the insects grow older they can be transferred more readily to new healthy plants. The results of experiments will be presented in table 11.

As shown in the table, all the infective nymphs tested retained their infectivity during all of the nymphal stages, the virus being retained during the process of moulting, and through the entire adult life or for a period of 50-90 days, without access of the virus. In this series of experiment also, most of the infective leafhoppers occasionally failed to produce infections in healthy rice plants. It appears that a period from 1 to 14 days must elapse before most of the infective nymphs become capable of producing infections in healthy plants although certain individuals may be infective immediately after the emergence from eggs. This admits several interpretations: either (1) the tiny newly emerged nymphs may be able only to transfer a dose of the virus insufficient to produce infection in a healthy rice plant, or (2) some developmental change or multiplication of the virus may occur within

the insect body before it is fully infective, or (3) the virus may migrate from the other parts of the body to the salivary glands and the anterior portion of alimentary tract before it is injected into the plant tissue through the proboscis.

5. *Virulence of the progeny of the infective leafhoppers*

As stated before, only a small portion of the leafhoppers reared on dwarf diseased rice plants were capable of transmitting the disease and most of the infective leafhoppers could produce infections in healthy plants when they were allowed to feed upon the plants less than one hour. This appeared to suggest the presence of "active" and "inactive" races of leafhoppers in the transmission of the disease as in the case of the leafhopper, *Cicadulina mbila* which acts as vector of the virus of maize streak.[214]

A few infective leafhoppers were introduced in an insect proof cage enclosing dwarf diseased rice plants. Each female leafhopper soon produced a numerous progeny. The younger leafhoppers, adult or nymphs just before the final moulting, thus reared on diseased rice plants were tested singly for their capability of transmitting the disease by means of the "single hopper culture method" described before. The results obtained will be shown in the following table.

TABLE 12. Results of transmission experiments with the progeny of infective leafhoppers, reared on diseased plants

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
e′ 1	male	Feb. 2, 1933	Feb. 25
2	"	"	Feb. 14
3	"	"	Feb. 17
4	"	"	Feb. 12
5	"	"	Feb. 15
6	nymph (subsequently ♀)	Mar. 6	Mar. 16
7	" (♀)	"	no infection
8	"	"	Mar. 18
9	" (♀)	"	Mar. 15
10	" (♀)	"	Mar. 13
11	"	"	"

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
e′ 12	nymph (subsequently ♀)	Mar. 6	Mar. 13
13	„ (♀)	„	Mar. 14
14	„ (♂)	„	Mar. 25
15	„	„	Mar. 18
16	„	„	Mar. 13
17	„	„	Mar. 12
18	„ (♂)	„	Mar. 17
19	„	„	Mar. 14
20	„ (♂)	„	Mar. 16
21	„ (♂)	„	„
22	„ (♂)	„	Mar. 19
23	„	Mar. 21	Apr. 2
24	„	„	Apr. 1
25	„	„	Apr. 2
26	„	„	Mar. 30
27	„	„	Apr. 5
28	„	„	Apr. 3
29	„ (♂)	„	„
30	„	„	Apr. 6
31	„	„	Apr. 4
32	„	„	Apr. 1
33	„	„	„
34	„ (♀)	„	„
35	„	„	„
36	„ (♂)	„	Apr. 2
37	„	„	Apr. 6
38	„	„	Mar. 30
39	„ (♀)	„	„
40	„	„	Apr. 2
41	„	„	Apr. 1
42	„	„	Apr. 3
43	„	„	Apr. 4
44	„	„	Apr. 6
45	„	„	Mar. 31
46	„	„	Apr. 1
47	„ (♂)	„	Apr. 2
48	„	May 19	May 28

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
e' 49	nymph (subsequently ♀)	May 19	June 5
50	„ (♀)	„	May 28
51	„ (♀)	„	May 27
52	„ (♂)	„	May 26
53	„ (♀)	„	June 3
54	„ (♂)	„	June 6
55	„ (♀)	„	June 5
56	„ (♀)	„	May 30
57	„ (♂)	May 26	June 10
58	„ (♂)	„	June 13
59	„ (♂)	„	„
60	„ (♂)	„	June 6
61	„ (♀)	„	June 24
62	„ (♀)	„	June 6
63	„ (♂)	„	June 16
64	„ (♂)	„	June 6
65	„ (♀)	„	June 8
66	„ (♀)	„	„
67	„ (♂)	„	June 6
68	„ (♂)	„	June 11
69	„ (♀)	„	June 24
70	„ (♀)	„	June 6
71	„ (♂)	„	June 8
72	„ (♂)	„	June 13
73	female	July 13	July 20
74	male	„	„ 19
75	„	„	„ 21
76	nymph	„	„ 24
77	„	„	„ 25
78	female	„	„ 18
79	„	Aug. 26	Sept. 2
80	„	„	„ 5
81	„	„	no infection
82	„	„	Sept. 2
83	„	„	„ 3
84	„	„	no infection
85	„	„	Sept. 9

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
c' 86	female	Aug. 26	no infection
87	,,	,,	Sept. 2
88	,,	,,	,, 20
89	male	,,	,, 10
90	,,	,,	,, 4
91	,,	,,	,, 2
92	,,	,,	,, 2
93	,,	,,	no infection
94	,,	,,	Sept. 4
95	,,	,,	,, 1
96	,,	,,	,, 6
97	,,	,,	,, 4
98	,,	,,	,, 4
99	,,	,,	,, 2
100	,,	,,	,, 4
101	,,	,,	,, 4
102	,,	,,	,, 20
103	,,	,,	,, 4
104	,,	,,	no infection
105	,,	,,	Sept. 3
106	,,	,,	,, 4
107	,,	,,	,, 1
108	nymph	,,	,, 1
109	,, (♀)	,,	,, 2
110	,, (♀)	,,	no infection
111	,,	,,	Sept. 4
112	,,	,,	,, 2
113	,,	,,	,, 6
114	male	,,	,, 2
115	female	Nov. 2	Nov. 16
116	,,	,,	,, 11
117	,,	,,	Dec. 14
118	,,	,,	no infection
119	male	,,	,,
120	female	,,	Nov. 11
121	,,	,,	,, 11
122	nymph (♀)	Nov. 9	,, 24
123	,,	,,	,, 23

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
e'124	nymph	Nov. 9	Nov, 18
125	„ (♀)	„	*no infection*
126	„	„	Nov. 24
127	„	„	„ 21
128	„ (♀)	„	„ 21
129	„ (♂)	„	„ 21
130	„ (♂)	„	„ 22
131	„ (♀)	„	„ 24
132	„	„	„ 24
133	„ (♀)	„	„ 18
134	„ (♀)	„	„ 26
135	„ (♂)	„	„ 24
136	„ (♀)	„	„ 27
137	„	„	„ 23
138	„ (♀)	„	„ 21
139	„ (♂)	„	Dec. 1
140	„	„	Nov. 19
141	„ (♀)	„	„ 21
142	„	„	„ 23
143	„ (♀)	„	„ 23
144	„ (♀)	„	Dec. 14
145	„	„	Nov. 28
146	„ (♀)	„	„ 21
147	„ (♀)	„	„ 21
148	„ (♀)	„	Dec. 14
149	„ (♂)	„	Nov. 23
150	„ (♀)	Nov. 25	Dec. 30
151	„ (♂)	„	„ 18
152	„ (♀)	„	„ 26
153	„	„	*no infection*
154	„	„	Dec. 13
155	„ (♀)	„	no infection
156	„	„	„
157	„	„	„
158	„	„	„
159	„ (♀)	„	Dec, 14
160	„ (♀)	„	„ 14
161	„	„	„ 15

Insect No.	Sex	The date of transfer of the insect to healthy plant	Appearance of the symptom
e' 162	nymph	Nov. 25	Dec. 27
163	,,	,,	,, 20
164	,,	,,	,, 11
165	,, (♀)	,,	,, 14
166	,,	,,	no infection
167	,, (♂)	,,	Dec. 11
168	,, (♀)	,,	,, 11
169	,,	,,	,, 15

As shown above, 153 individuals out of 169 leafhoppers tested produced infections in healthy rice plants, which began to show the symptom 6–29 days, mostly 9–13 days after the insects were transferred to these plants. In other words almost all the progeny of infective leafhoppers were viruliferous when they had been reared upon diseased plants whereas a large proportion of leafhoppers from the other colonies bred on diseased plants were free from virus. This admits two interpretations: either the offspring of infective leafhoppers may have "inherited" the virus from their parents or they may have the predisposition to carry the virus readily after feeding upon diseased rice plants.

In parallel to the experiments above mentioned, a series of experiments were started to determine whether the infective power is "inheritable" to the offspring by breeding successive generations from infective parents or from crosses between infective females and uninfective males and vice versa.

A pair of male and female leafhoppers, either one or both infective, were confined on a young healthy rice plant in a glass tube and allowed to remain there for one day. Subsequently they were transferred to new healthy rice plants every day during which period oviposition occurred. The precaution was taken to confine single nymphs on rice plants in separate glass tubes and to allow them to remain there until they grew into adults in order to obtain female leafhoppers which had never copulated. The eggs are laid in the leafsheath at a few spots near the ground and thrust transversely into the tissue lying one under the other. A few days after oviposition the spot where the eggs are deposited becomes easily discernible by its slightly discolored and more

or less raised surface. The eggs hatch in about 10 days. The tiny nymphs just emerged from eggs were transferred to healthy rice plants taking the necessary precaution so that they had no opportunity to feed upon the plants on which the eggs had been deposited and which might have been affected with the disease in consequence of the infestation of the infective parents. Daily observations were continued as the hatching of the eggs approached and the nymphs which had just emerged from the eggs were transferred singly to a young healthy rice plant in a glass tube by means of a small camel's hair brush. The eggs usually hatch in the early morning and in a similar manner to that described by STAHL and CARSNER (1918)[206] and also by SEVERIN (1921)[186] in respect to the beet leafhopper, *Eutettix tenella*. Keeping the plants which harbour the eggs in a dark and cool place the hatching of eggs could be retarded to some extent. When such plants were placed in the sunlight, some of these eggs immediately began to hatch out. Another method of transfer of the nymphs is as follows: the leafsheath with eggs is cut off 4–5 cm. long and leaned against healthy rice plants in a glass tnbe. When the eggs hatch the tiny nymphs immediately migrate to the healthy plants crawling up the stems and they later can be easily transferred singly to a young healthy rice plant in a glass tube. The results of experiments will be shown in the following tables.

TABLE 13. Virulence of the progeny of infective leafhoppers

Infective female × uninfective male

No. e 279 (♀) × a (♂)

Insect No.	Date of emergence from egg	Date of appearance of symptom	Sex of insect (when matured)
e 279–1	Oct. 29' 32	Nov. 21	female
e 279–2	"	24	"
e 279–3	"	30	"
e 279–4	"	22	"
e 279–5	"	24	"
e 279–6	"	"	"
e 279–7	"	"	male
e 279–8	"	16	female
e 279–9	"	18	"

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Insect No.	Date of emergence from egg	Date of appearance of symptom	Sex of insect (when matured)
e 279-10	Oct. 30	Nov. 25	prematurely died
e 279-11	„	„	*male*
e 279-12	„	21	prematurely died
e 279-13	31	28	female
No. e 151-4 (♀) × b (♂)			
e 151-4-1	Nov. 4	Dec. 23	prematurely died
e 151-4-2	„	25	„
e 151-4-3	„	no infection	female
e 151-4-4	„	Dec. 14	„
e 151-4-5	5	15	prematurely died
e 151-4-6	„	2	female
e 151-4-7	6	12	„
e 151-4-8	„	25	„
e 151-4-9	„	5	male
e 151-4-10	Nov. 7	Dec. 15	prematurely died
e 151-4-11	„	5	„
e 151-4-12	8	Nov. 26	female
e 151-4-13	„	Dec. 29	„
e 151-4-14	9	7	male
e 151-4-15	10	14	female
e 151-4-16	„	20	„
No. e 151-1 (♀) × c (♂)			
e 151-1-1	Nov. 5	no infection	female
e 151-1-2	„	Dec. 2	„
e 151-1-3	„	no infection	male
e 151-1-4	6	Dec. 2	female
e 151-1-5	7	no infection	„
e 151-1-6	„	Jan. 2	„
e 151-1-7	9	Dec. 8	male
e 151-1-8	„	no infection	female
e 151-1-9	12	„	male
e 151-1-10	„	Dec. 23	female
e 151-1-11	13	30	male

Insect. No.	*Date of emergence* from egg	*Date of appear-* ance of symptom	*Sex of insect* (when matured)
e 151-1-12	15	no infection	female
e 151-1-13	"	Dec. 29	male
e 151-1-14	16	no infection	female
e 151-1-15	"	"	male
e 151-1-16	"	Dec. 17	female
e 151-1-17	18	no infection	"
e 151-1-18	"	Jan. 7	prematurely died
e 151-1-19	20	Dec. 17	male
e 151-1-20	"	13	female
e 151-1-21	"	"	"
e 151-1-22	"	19	male
e 151-1-23	21	10	"
e 151-1-24	"	20	female
e 151-1-25	22	Jan. 9	"
e 151-1-26	"	Dec. 14	"

No. e 151-4-4 (♀) × d (♂)

e 151-4-4-1	Jan. 16	no infection	male
e 151-4-4-2	"	"	prematurely died
e 151-4-4-3	"	"	"
e 151-4-4-4	"	"	female
e 151-4-4-5	"	"	male
e 151-4-4-6	"	"	female

No. e 279-4 (♀) × e (♂)

e 279-4-1	Jan. 31	Feb. 20	prematurely died
e 279-4-2	"	15	"
e 279-4-3	"	23	"
e 279-4-4	Feb. 1	20	male
e 279-4-5	"	17	female
e 279-4-6	"	no infection	prematurely died
e 279-4-7	"	"	"
e 279-4-8	2	Feb. 12	"
e 279-4-9	"	20	"
e 279-4-10	"	23	"

Insect. No.	Date of emergence from egg	Date of appearance of symptom	Sex of insect (when matured)
e 279-4-11	Feb. 2	Feb. 15	female
e 279-4-12	3	Mar. 1	prematurely died
e 279-4-13	"	Feb. 24	female
e 279-4-14	"	19	male
e 279-4-15	"	15	"
e 279-4-16	4	23	female
e 279-4-17	"	Mar. 1	prematurely died
e 279-4-18	"	Feb. 24	female
e 279-4-19	"	23	male
e 279-4-20	"	21	prematurely died
e 279-4-21	"	Mar. 7	"
e 279-4-22	5	Feb. 21	"
e 279-4-23	"	"	"
e 279-4-24	"	22	male
e 279-4-25	7	27	prematurely died
e 279-4-26	8	Mar. 10	female
e 279-4-27	"	1	prematurely died
e 279-4-28	"	3	female
e 279-4-29	10	16	prematurely died
e 279-4-30	"	1	female
e 279-4-31	12	5	prematurely died
e 279-4-32	"	3	male
e 279-4-33	14	1	prematurely died
e 279-4-34	"	2	"
e 279-4-35	"	Feb. 27	female

No. e 279-1 (♀) × f (♂)

e 279-1-1	Feb. 11	no infection	
e 279-1-2	12	"	
e 279-1-3	"	"	
e 279-1-4	"	"	
e 279-1-5	14	"	
e 279-1-6	"	"	

Insect. No.	Date of emergence from egg	Date of appearance of symptom	
e 279-1-7	Feb. 14	no infection	
e 279-1-8	,,	,,	
e 279-1-9	16	,,	
e 279-1-10	18	,,	
e 279-1-11	20	,,	
e 279-1-12	,,	,,	
e 279-1-13	,,	,,	
e 279-1-14	,,	,,	
e 279-1-15	,,	,,	
e 279-1-16	,,	,,	
e 279-1-17	,,	,,	
e 279-1-18	,,	,,	
e 279-1-19	,,	,,	
e 279-1-20	,,	,,	
e 279-1-21	,,	,,	
e 279-1-22	,,	,,	
e 279-1-23	24	,,	
e 279-1-24	,,	,,	
e 279-1-25	,,	,,	
e 279-1-26	,,	,,	
e 279-1-27	,,	,,	

No. e 151-1-16 (♀) × g (♂)

Insect. No	Date of emergence from egg	Length of feeding period	Result
e 151-1-16-1	Feb. 5	30 days	no infection
e 151-1-16-2	,,	10	,,
e 151-1-16-3	,,	30	,,
e 151-1-16-4	,,	,,	,,
e 151-1-16-5	,,	10	,,
e 151-1-16-6	,,	20	,,
e 151-1-16-7	6	30	,,
e 151-1-16-8	,,	,,	,,
e 151-1-16-9	,,	10	,,
e 151-1-16-10	,,	30	,,
e 151-1-16-11	,,	,,	,,
e 151-1-16-12	,,	10	,,
e 151-1-16-13	7	30	,,
e 151-1-16-14	,,	,,	,,

Insect. No.	Emergence from egg	Length of feeding period	Result
e 151-1-16-15	Feb. 7	30 days	no infection
e 151-1-16-16	8	5	„
e 151-1-16-17	„	30	„
e 151-1-16-18	„	20	„
e 151-1-16-19	„	„	„
e 151-1-16-20	„	„	„
e 151-1-16-21	„	„	„
e 151-1-16-22	„	„	„
e 151-1-16-23	9	10	„
e 151-1-16-24	„	„	„
e 151-1-16-25	10	30	„
e 151-1-16-26	„	10	„
e 151-1-16-27	„	„	„
e 151-1-16-28	11	30	„
e 151-1-16-29	„	„	„
e 151-1-16-30	„	5	„

No. e′51 (♀) × h (♂)

Insect No.	Emergence from egg	Appearance of symptom	
e′ 51-1	June 30	July 15	
e′ 51-2	„	11	
e′ 51-3	„	12	
e′ 51-4	„	11	
e′ 51-5	July 8	25	
e′ 51-6	9	22	
e′ 51-7	10	25	
e′ 51-8	11	24	
No. e 279-4-5-3-3 (♀) × i (♂)			
e 279-4-5-3-3-1	July 1	July 15	
e 279-4-5-3-3-2	9	31	
e 279-4-5-3-3-3	„	„	
e 279-4-5-3-3-4	„	29	
e 279-4-5-3-3-5	„	Aug. 16	
No. e′62 (♀) × j (♂)			
e′ 62-1	July 7	July 26	
e′ 62-2	„	23	

No. e′ 69 (♀) × k (♂)

Insect No.	Emergence from egg	Appearance of symptom	
e′ 69-1	July 11	no infection	
e′ 69-2	,,	,,	
No. e′ 65 (♀) × l (♂)			
e′ 65-1	July 15	Aug. 2	
No. e′ 61 (♀) × m (♂)			
e′ 61-1	July 17	Aug. 10	
e′ 61-2	,,	no infection	
e′ 61-3	,,	,,	
e′ 61-4	19	,,	
e′ 61-5	,,	Aug. 4	
e′ 61-6	,,	no infection	
e′ 61-7	,,	Aug. 6	
e′ 61-8	,,	*no infection*	
e′ 61-9	,,	Aug. 6	
e′ 61-10	,,	,,	
e′ 61-11	20	no infection	
e′ 61-12	,,	Aug. 5	
No. e′ 131 (♀) × n (♂)			
e′ 131-1	Jan. 1 '34	Jan. 30	
e′ 131-2	,, 12	,, 29	
e′ 131-3	,, 13	Feb. 7	
e′ 131-4	,, ,,	,, 14	
e′ 131-5	,, 14	Jan. 29	

Infective female × infective male

No. e 279-4-5 (♀) × No. e 279-4-4 (♂)

Insect No.	Date of emergence from egg	Date of appearance of symptom	Sex of insect (when matured)
e 279-4-5-1	Mar. 26	Apr. 6	male
e 279-4-5-2	27	no infection	,,
e 279-4-5-3	,,	,,	female
e 279-4-5-4	,,	Apr. 6	male
e 279-4-5-5	,,	10 (?)	,,
e 279-4-5-6	Apr. 2	10 (?)	,,
e 279-4-5-7	4	23	female

No. e′34 (♀) × No. e′29 (♂)

Insect No.	Date of emergence from egg	Date of appearance of symptom	Sex of insect (when matured)
e′34-1	Apr. 30	May 16	female
e′34-2	″	14	p. d. *
e′34-3	May 3	22	male
e′34-4	9	20	″
e′34-5	22	June 14	female
e′34-6	25	21	male
e′34-7	June 8	23	″
e′34-8	10	25	″
e′34-9	″	24	″
e′34-10	″	26	female
e′34-11	″	no infection	″
e′34-12	19	July 6	″
e′34-13	″	7	male
e′34-14	″	″	female

No. e′39 (♀) × No. e′36 (♂)

e′39-1	May 1	May 13	male
e′39-2	″	16	p. d.
e′39-3	″	18	male
e′39-4	″	15	″
e′39-5	10	29	″
e′39-6	21	June 14	female
e′39-7	″	5	″
e′39-8	″	10	male

No. e′49 (♀) × No. e′68 (♂)

e′49-1	July 8	July 25	p. d.
e′49-2	10	27	female
e′49-3	″	24	p. d.
e′49-4	″	″	″

No. e′53 (♀) × No. e′58 (♂)

e′53-1	July 8	July 27	male

* prematurely died

uninfective female × infective male

No. of insects tested	Date of emergence from egg	Length of feeding period	No. of infective leafhoppers
a (♀) × No. e 151-1-22 (♂)			
2	Jan. 25'33	31 days	0
2	26	30	0
5	27	29	0
b (♀) × No. e 151-1-13 (♂)			
10	Feb. 7	33	0
1	8	32	0
2	9	31	0
5	10	30	0
1	12	28	0
c (♀) × No. e 151-1-13 (♂)			
5	Feb. 14	31	0
d (♀) × No. e' 47 (♂)			
5	Apr. 30	30	0
3	May. 4	„	0
1	5	„	0
e (♀) × No. e 279-4-32 (♂)			
5	May 13	30	0
3	15	„	0
6	17	„	0
f (♀) × No. e' 64 (♂)			
4	July 1	30	0
g (♀) × No. e' 72 (♂)			
3	July 2	30	0
h (♀) × No. e' 129 (♂)			
2	Dec. 29	52	0
2	„ 30	51	0
7	„ 31	50	0
1	Jan. 5'34	45	0
1	„ 7	43	0

No. of insects tested	Date of emergence from egg	Length of feeding period	No. of infective leafhoppers
3	Jan. 10 '34	40	0
4	„ 15	35	0
2	„ 19	31	0
i (♀) × No. e' 129 (♂)			
3	Jan. 8 '34	42	0
1	„ 13	37	0
5	„ 15	35	0
3	„ 21	29	0
j (♀) × *No. e'* 135 (♂)			
5	Dec. 21	60	0
2	„ 28	53	0
3	Jan. 1 '34	49	0
k (♀) × No. e' 151 (♂)			
3	Dec. 27	54	0
4	„ 28	53	0
3	„ 29	52	0
1	„ 31	50	0
3	Jan. 1 '34	49	0
l (♀) × No. e' 151 (♂)			
1	Jan. 3 '34	47	0
2	„ 4	46	0
2	„ 8	42	0
3	„ 9	41	0
1	„ 14	36	0
m (♀) × No. e' 167 (♂)			
3	Dec. 29	53	0
4	„ 30	51	0
n (♀) × No. e' 167 (♂)			
1	Dec. 29	52	0
1	„ 31	50	0
3	Jan. 1'34	49	0
2	„ 4	46	0

The data in the above table will be shown summarized as follows.

TABLE 14. Summarized results of the experiments with the progeny of infective leafhoppers

Parents		No. of progeny tested	No. of progeny infective
female	male		
infective female × infective male			
e 279–4–5	e 279–4–4	7	5
e′ 34	e′ 29	14	13
e′ 39	e′ 36	8	8
e′ 49	e′ 68	4	4
e′ 53	e′ 58	1	1
infective female × uninfective male			
e 279	a	13	13
e 151–4	b	16	15
e 151–1	c	26	17
e 151–4–4	d	6	0
e 279–4	e	35	33
e 279–1	f	27	0
e 151–1–16	g	30	0
e′ 51	h	8	8
e 279–4–5–3–3	i	5	5
e′ 62	j	2	2
e′ 69	k	2	0
e′ 65	l	1	1
e′ 61	m	12	6
e′ 131	n	5	5
uninfective female × infective male			
a	e 151–1–22	9	0
b	e 151–1–13	19	0
c	”	5	0
d	e′ 47	9	0
e	e 279–4–32	14	0
f	e′ 64	4	0
g	e′ 72	3	0
h	e′ 129	22	0
i	”	12	0
j	e′ 135	10	0
k	e′ 151	14	0
l	”	9	0
m	e′ 167	7	0
n	”	7	0

As shown in above table, the majority of the offspring from the *infective parents proved to be viruliferous and the progeny from the* crosses between infective females and uninfective males were either viruliferous or free from the virus whereas those from the crosses between uninfective females and infective males were entirely free from virus. It appears that the eggs are not affected by the virus after they have been deposited in the leafsheath but probably at an early stage of their development in the ovary of the maternal insect body, because in no case has it been observed that infective progeny emerged out of the eggs from the uninfective females which had been laid in the leafsheaths of diseased plants. It is worthy of note that some individuals of the progeny from the infective females were viruliferous while the others from the same parents were apparently free from the virus. This seems to indicate that all the ova produced in an ovary are not always affected by the virus.

Although a number of virus diseases of plants have been demonstrated to be transmissible by the agency of certain insects, in no case has it been shown that the virus is transmitted through the eggs of an insect carrier. McCLINTOCK and SMITH (1918)[129] reported that the virus of spinach blight (or spinach mosaic) was transmitted from infective aphids to their viviparously produced progeny up to the 4th generation. This is the only evidence yet recorded that indicates the possible transmission of virus from insect vectors to their offspring. HOGGAN (1933),[80] however, has not been able to confirm their conclusion. CARSNER and STAHL (1924)[34] state that "the ability to produce the curly top of beet is not inherent" in the leafhopper, *Eutettix tenella* BAKER. Aster yellows is, according to KUNKEL (1926),[111] not transmitted through the eggs of the insect carrier. STOREY (1928)[212] also reports in his work on maize streak disease that "the eggs laid by infective leafhoppers (*Cicadulina mbila*) produced uninfective progeny."

Dwarf disease of rice plant, therefore, occupies a unique position among the virus diseases of plants for the reason of its transmission through the eggs of the insect carrier.

According to MURATA (1931),[149] ONUKI and MURATA, formerly entomologists in the Imperial Agricultural Experiment Station, were probably the first to notice that the offspring of infective leafhoppers were capable of producing dwarf disease in healthy rice plants. In 1902? they found that certain leafhoppers emerged from the eggs which had been taken from Shiga Prefecture where this trouble was prevalent

at that time, produced infections in healthy rice plants. However, they gave no detailed account of it. The idea generally prevailing at that time was that the disease was due to the infestation of leafhoppers and accordingly these entomologists considered that the capability of producing dwarf disease was a characteristic specific to the leafhoppers native to the Prefecture of Shiga because all the leafhoppers of the same species taken in the vicinity of Tôkyo failed to produce infections in healthy rice plants. Later, however, they found that even uninfective leafhoppers from Tôkyo became infective after feeding upon dwarf diseased rice plants as stated before. MURATA (1915)[148] briefly stated that the capability of producing dwarf disease of rice plant was transmitted from infective parents to their progeny through 3 or 4 generations but he presented no experimental procedures nor results which are considered to be sufficient ground for inducing such very significant conclusions.

At any rate these entomologists were evidently in ignorance of the fact that they were dealing with a virus disease in dwarf disease of rice plant and that the virus may be transmitted through the eggs of the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL.

6. *Inoculation of rice plants with the macerated tissues of infective leafhoppers*

On April 5, 1933, about one hundred infective leafhoppers, a majority of them being nymphs in the 3rd or 4th instars, were ground in a mortar, adding a few drops of tap water. With the macerated tissues of leafhoppers thus prepared, 20 young rice plants were inoculated. Two inoculations were made on each plant, one on the leaf and the other at the base of stem. The inoculum was placed with a sterile scalpel on the desired spot and 30 pricks were made through the macerated tissues and body fluid of infective leafhoppers with a sterile needle, thus working the latter into the tissue. The inoculated plants were 2 weeks old plants of "Aikoku"-variety. All the inoculated plants remained healthy.

May 28, a similar experiment was carried out inoculating 30 young rice plants with the macerated tissues and body fluid of infective leafhoppers. The inoculum was prepared by grinding about 70 leafhoppers in a mortar adding a few drops of 0.85% saline solution. The procedure of inoculation was exactly the same as mentioned above. No symptoms of the disease appeared on any of the inoculated plants.

As a consequence of these inoculation experiments it appears that dwarf disease of rice plant is not transmitted to healthy plants by means of the macerated tissues and body fluid of infective leafhoppers.

7. *Acquisition of infective power by the leafhopper*

As stated before, the larger portion of the adult leafhoppers which had been reared upon dwarf diseased rice plants were entirely free from the virus. Accordingly the writer was naturally led to the supposition that most leafhoppers would not acquire infective power when the feeding period upon diseased plants was reduced to several days.

Non-viruliferous leafhoppers were allowed to feed upon dwarf diseased rice plants in insect proof cages for a definite length of time and transferred singly to a healthy rice seedling plant in a glass tube as described before, to test their infectivity. The effect was also tested of groups of these leafhoppers upon healthy rice plants in insect proof cages. Generally male leafhoppers were used instead of females in order to avoid egg deposition.

a) Resnlts of experiments carried out in 1927

The non-viruliferous leafhoppers used in this series of experiments were collected on rice plants in the vicinity of Tottori where dwarf disease has never been found to occur.

TABLE 15. Acquisition of infective power by the leafhopper (glass tube experiments in 1927)

Date	No. of leafhoppers tested	Sex	Length of feeding period		No. of infective insects
			on diseased plant	on healthy plant	
Oct. 12	3	♂	1 day	3 days	0
7	2	"	"	5	0
Sept. 30	3	"	"	7	0
10	3	"	"	14	0
1	6	"	7	1	0
4	6	"	"	"	0
1	6	"	"	3	0

b) Results of experiments carried out in 1928

The leafhoppers used in these experiments were the progeny of healthy leafhoppers which had been maintained on healthy rice plants since the previous season.

TABLE 16. Acqnisition of infective power by the leafhopper (glass tube experiments in 1928)

Date	No. of leafhoppers tested	Sex	Length of feeding period		No. of infective insects
			on diseased plant	on healthy plant	
July 15	6	♂	3 days	16 days	0
Aug. 31	7	,,	10	13	0
Apr. 4	3	♀	30	3	0
Mar. 30	9	♂	,,	7	0
,,	9	♀	,,	,,	0
Sept. 10	5	♂	10	16	0
11	6	,,	20	20	0
,,	8	,,	30	20	0

c) Results of experiments carried out in 1930–31

On the fourteenth of April, 80 or 90 leafhoppers were collected on *Astragalus sinicus* L. growing on the rice fields in the vicinity of Utsunomiya and two days later about half the number were introduced into insect proof cages enclosing healthy rice plants in the green house. The progeny of these insects were maintained on healthy rice plants to obtain non-viruliferous colonies of leafhoppers at the desired moment during the period of experiment. They were allowed to feed upon diseased plants for a definite length of time ranging from 1 to 70 days and then confined either individually on a single healthy rice plant in a glass tube or in a group on healthy plants in insect proof cages. As a rule, these insects were allowed to remain on the healthy plants until they died. The results of experiments will be shown below.

TABLE 17. Acquisition of infective power by the leafhopper (glass tube experiments in 1930–31)

Date	No. of *insects* tested	*Sex*	*Length of feeding period* on diseased *plant*	Temp.	on healthy *plant*	Temp.	No. of *infective* insects
May 5	2	♂	1 day	15–25°C	41–50 days	14–33°C	0
8	4	,,	,,	17–28	31–40	,,	0
16	7	,,	,,	19–27	21–30	13–33	0
20	2	,,	,,	13–42	,,	13–32	0
June 10	5	,,	,,	13–29	71–80	18–33	0
12	4	,,	,,	13–33	61–70	21–36	0
18	5	,,	,,	14–30	71–80	,,	0
,,	5	,,	3	13–31	61–70	22–35	0
Apr. 28	1	,,	7	16–32	11–20	16–28	0
June 11	5	,,	,,	14–32	61–70	22–36	0
Apr. 20	4	,,	15	16–32	11–20	17–29	0
June 18	11	,,	,,	15–30	51–60	21–36	0
,,	5	,,	20	,,	41–50	22–36	0
Aug. 1	4	,,	,,	22–36	21–30	17–32	0
,,	2	,,	,,	,,	41–50	16–36	0
,,	1	,,	,,	,,	51–60	,,	0
Oct. 10	1	,,	,,	13–34	21–30	13–30	0
,,	1	,,	,,	,,	51–60	16–28	0
Sept. 30	1	,,	30	13–36	1–10	15–33	0
,,	3	,,	,,	,,	11–20	13–31	0
,,	4	,,	,,	,,	21–30	,,	0
,,	1	,,	,,	,,	41–50	14–30	0
,,	1	,,	,,	,,	51–60	16–28	0
Nov. 19	1	,,	,,	16–29	11–20	16–29	0
,,	1	,,	,,	,,	31–40	16–31	1 (Jan. 27)
,,	2	,,	,,	,,	41–50	17–31	0
,,	1	,,	,,	,,	51–60	,,	0
,,	1	n	,,	,,	21–30	16–30	0
,,	1	,,	,,	,,	31–40	16–31	0
,,	1	,,	,,	,,	41–50	17–31	0
,,	2	,,	,,	,,	51–60	,,	0
Dec. 7	3	♂	,,	,,	11–20	,,	0
,,	1	,,	,,	,,	21–30	17–32	0
,,	2	,,	,,	,,	31–40	18–33	0
,,	1	,,	,,	,,	41–50	,,	0
Nov. 19	7	,,	50	,,	21–30	17–31	2 (Feb. 18, Feb. 2)
,,	2	,,	,,	,,	31–40	,,	0
,,	1	,,	,,	,,	41–50	17–32	0
Dec. 16	1	,,	,,	17–30	1–10	17–34	0
,,	3	,,	,,	,,	11–20	18–35	1 (Feb. 20)
,,	3	,,	,,	,,	21–30	,,	1 (Mar. 1)
,,	2	,,	,,	,,	31–40	,,	0

n stands for nymph.

Date	No. of insects tested	Sex	Length of feeding period				No. of infective insects
			on diseased plant	Temp.	on healthy plant	Temp.	
Dec. 16	1	♂	50 days	17–30°C	51–60 days	18–36°C	0
17	2	,,	,,	,,	1–10	,,	0
,,	2	,,	,,	,,	11–20	,,	0
,,	1	,,	,,	,,	21–30	,,	0
Nov. 19	4	,,	70	16–30	1–10	17–36	0
,,	2	,,	,,	,,	11–20	18–35	0
,,	1	,,	,,	,,	21–30	,,	0
,,	1	,,	,,	,,	31–40	,,	0
Dec. 16	1	♂	,,	17–32	1–10	,,	0
,,	1	,,	,,	,,	11–20	,,	0
,,	1	,,	,,	,,	21–30	,,	0
,,	7	,,	,,	,,	31–40	,,	0

TABLE 18. *Acquisition of infective power by the leafhopper*
(cage experiments in 1930)

Date	No. of insects in each cage	No. of plants in each cage	Length of feeding period				No. of infective insects
			on diseased plant		on healthy plant		
Sept. 25	30 (♂)	2	10 days	(15–36°C)	3–62 days	(14–32°C)	1*
,,	20 ,,	2	,,	,,	,,	,,	1*
12	10 ,,	2	15	,,	11–34	(13–36)	0
,,	6 ,,	2	,,	,,	11–13	,,	0
25	6 ,,	2	,,	(14–38)	5– 7	(8–35)	0
2	5 ,,	2	20	(16–36)	1– 5	(14–35,)	0
29	15 ,,	3	30	(13–36)	2–19	(13–31)	0
,,	6 ,,	2	,,	,,	2–15	,,	0
Dec. 7	10 ,,	10	,,	(16–29)	6–59	(18–34)	0

* On Dec. 6, sixty-two days after the transfer of the insects to healthy rice plants, only one leafhopper was found alive in each of the two cages labelled A and B. These insects were then confined singly in a glass tube enclosing a healthy rice plant. The insect from cage A was found dead on Dec. 10 and the other on Dec. 30. On Dec. 26, distinct symptoms of dwarf disease began to appear on both rice plants which had been subjected to the infestation of these insects in glass tubes. Prior to this, one of the plants in cage A began to show the symptoms of disease on Dec. 21 whereas the other plant in the same cage and the plants in cage B remained healthy until Jan. 16. The evidence seems to indicate that the above mentioned leafhopper in cage B remained uninfective during a period as long as 62 days at least after it had picked up the virus from dwarf diseased rice plants. In other words the incubation period of the virus in this insect body was more than 62 days under the writer's experimental conditions.

d) Results of experiments carried out in 1931

The leafhoppers used in these experiments were the progeny of non-viruliferous leafhoppers which had been cultured since the previous year in insect proof cages enclosing healthy rice plants.

TABLE 19. Acquisition of infective power by the leafhopper (glass tnbe experiments in 1931)

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant	Temp.	on healthy plant	Temp.	
Aug. 20	1 (♂)	5 days	20–37°C.	11–20 days	16–35°C.	0
„	2 „	„	„	21–30	„	0
„	1 „	„	„	41–50	„	0
„	2 „	„	„	51–60	16–34	0
Sept. 5	5 „	„	16–35	21–30	14–35	0
„	2 „	„	„	31–40	15–34	0
„	2 „	„	„	41–50	„	0
„	3 „	„	„	51–60	16–34	0
„	3 „	„	„	61–70	17–34	0
„	3 (♀)	„	„	21–30	14–35	0
„	4 „	„	„	31–40	15–34	0
„	6 „	„	„	41–50	„	0
„	2 „	„	„	51–60	16–34	0
„	1 „	„	„	61–70	17–34	0
„	2 „	„	„	71–80	„	0
„	1 (♂)	10	16–36	21–30	14–33	0
„	2 „	„	„	31–40	15–33	0
„	2 „	„	„	61–70	17–33	0
„	1 (♀)	„	„	11–20	14–33	0
„	1 „	„	„	21–30	„	0
„	4 „	„	„	31–40	„	0
„	4 „	„	„	41–50	15–33	0
„	2 „	„	„	71–80	13–32	0
Aug. 24	3 (♂)	30	15–35	11–20	15–33	0
„	7 „	„	„	21–30	14–33	0
„	4 „	„	„	31–40	15–33	0
„	6 „	„	„	41–50	17–34	0
Sept. 17	2 „	„	14–32	1–10	19–34	0
„	1 „	„	„	11–20	18–35	0
„	6 „	„	„	21–30	19–34	0
„	4 „	„	„	31–40	20–34	0

TABLE 20. Acquisition of infective power by the leafhopper (cage experiments in 1931)

Date	No. of insects in each cage	No. of plants in each cage	Length of feeding period		No. of infective insect
			on diseased plant	on healthy plant	
Aug. 14	20 (♂)	20	1 day (20–35°C)	11–39 days (17–36°C)	0
„	19 „	20	3 (21–39)	9–19 (17–35)	0
17	20 (n)	20	„ (22–40)	11–49 (15–35)	0
18	35 „	20	5 („)	8–58 („)	0
31	17 (♂)	17	„ (17–37)	3–27 („)	0
May 22	*9 „*	*9*	*10 (16–31)*	*2–39 („)*	*0*
June 2	10 „	10	„ (10–31)	9–55 („)	0
Aug. 20	20 „	20	„ (15–35)	9–51 (16–34)	0
Sept. 5	17 „	17	„ (16–36)	10–40 (15–33)	0
Aug. 20	39 (♀)	20	30 (16–35)	2–45 (16–33)	0

e) Results of experiments carried out in 1932–33

On April 13, about two hundred nymphs of *Nephotettix apicalis* var. *cincticeps* were collected from *Astragalus sinicus* L. growing on the rice fields in the vicinity of Shiraoka near Ômiya. About 100 insects were confined in insect proof cages enclosing healthy rice plants. Almost all these leafhoppers proved free from virus and their progeny were kept in culture continuously on healthy rice plants in order to obtain non-viruliferous leaf hoppers at the desired moment.

TABLE 21. Acquisition of infective power by the leafhopper (glass tnbe experiments in 1932–33)

Date	No. of insects tested	*Length of feeding period*				No. of infective insects
		on diseased* plant	Temp.	on healthy plant	Temp.	
June 7	1 (♂)	5 days	14–30°C	1–10 days	15–33°C	0
„	3 „	„	„	11–20	16–33	0
„	9 (n)	„	„		„	0
„	1 (♂)	„	„	21–30	17–33	0
„	9 (n)	„	„	„	„	0
„	3 (♂)	„	„	31–40	18–33	0
„	3 (n)	„	„	„	„	0
„	1 (♂)	„	„	41–50	„	0

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant	Temp.	on healthy plant	Temp.	
June 7	6 (n)	5 days	14–30°C	41–50 days	18–33°C	0
„	1 (♂)	„	„	51–60	„	0
„	2 (n)	„	„	„	„	0
„	1 (♂)	„	„	„	„	0
June 23	1 (♀)	„	17–32	1–10	16–33	0
„	2 (♀)	„	„	11–20	17–33	0
„	2 (♀)	„	„	31–40	18–33	0
June 12	2 (♂)	10	16–33	1–10	17–33	0
„	1 (n)	„	„	„	„	0
„	1 (♂)	„	„	11–20	„	0
„	4 (n)	„	„	„	„	0
„	1 (♂)	„	„	21–30	17–32	0
„	4 (n)	„	„	„	„	0
„	5 (♂)	„	„	31–40	18–33	0
„	3 „	„	„	41–50	„	0
„	2 (n)	„	„	„	„	0
„	3 (♂)	„	„	51–60	18–34	0
„	1 (n)	„	„	61–70	18–33	0
„	1 „	„	„	71–80	„	0
„	1 „	„	„	81–90	„	0
June 23	1 (♂)	„	17–33	1–10	18–34	0
„	1 „	„	„	11–20	18–33	0
„	1 „	„	„	41–50	19–35	0
„	2 (♀)	„	„	„	„	0
„	1 „	„	„	51–60	„	0
Aug. 9	4 (♂)	„	21–38	1–10	18–36	1 (Aug. 31)
„	2 „	„	„	11–20	18–35	0
„	1 „	„	„	41–50	16–32	0
Aug. 19	8 „	„	18–37	1–10	19–32	0
„	9 „	„	„	11–20	18–32	1 (Sept. 14)
Sept. 14	1 „	„	11–32	1–10	13–32	0
„	3 „	„	„	11–20	13–33	0
„	5 „	„	„	21–30	13–34	0
„	3 „	„	„	31–40	14–34	0
„	5 „	„	„	41–50	15–34	0
„	1 „	„	„	51–60	18–33	0
Oct. 1	7 „	„	12–33	1–10	14–35	0

Date		No. of insects tested	Length of feeding period				No. of infective insects
			on diseased plant	Temp.	on healthy plant	Temp.	
Oct.	1	6 (♂)	10 days	12–33°C	11–20 days	18–34°C	0
	,,	3 ,,	,,	,,	21–30	19–34	0
	,,	2 ,,	,,	,,	31–40	,,	0
	,,	4 ,,	,,	,,	41–50	,,	0
Nov.	6	4 (n)	15	19–30	21–30	20–30	0
	,,	4 ,,	,,	,,	31–40	,,	0
	,,	2 ,,	,,	,,	41–50	,,	0
	,,	2 ,,	,,	,,	51–60	,,	0
	,,	5 ,,	,,	,,	61–70	,,	0
	,,	2 ,,	,,	,,	71–80	,,	0
June	24	2 (♂)	20	17–32	1–10	18–34	0
	,,	2 (n)	,,	,,	,,	,,	0
	,,	1 (♂)	,,	,,	11–20	20–35	0
	,,	7 (n)	,,	,,	,,	,,	0
	,,	2 ,,	,,	,,	21–30	20–36	0
	,,	5 (♂)	,,	,,	31–40	,,	0
	,,	3 (n)	,,	,,	,,	,,	0
	,,	1 (♂)	,,	,,	41–50	19–36	0
	,,	2 (n)	,,	,,	,,	,,	0
	,,	1 ,,	,,	,,	51–60	,,	0
	,,	2 ,,	,,	,,	61–70	19–34	0
July	16	1 (♂)	,,	20–36	1–10	20–35	0
	,,	1 ,,	,,	,,	11–20	21–35	0
Aug.	5	9 ,,	,,	19–36	1–10	18–33	0
	,,	7 ,,	,,	,,	11–20	,,	0
	,,	1 ,,	,,	,,	21–30	,,	0
	,,	2 ,,	,,	,,	31–40	16–32	0
	,,	1 ,,	,,	,,	41–50	15–32	0
Aug.	25	3 ,,	,,	18–33	11–20	11–32	0
	,,	1 ,,	,,	,,	21–30	13–32	0
	,,	1 ,,	,,	,,	31–40	12–33	0
July	3	9 ,,	30	,,	11–20	20–36	0
	,,	2 ,,	,,	,,	21–30	19–36	0
	,,	3 ,,	,,	,,	31–40	19–35	0
Oct.	25	1 (♀)	,,	19–32	11–20	18–31	0
	,,	1 (n)	,,	,,	,,	,,	0
	,,	1 (♂)	,,	,,	21–30	20–31	0

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant	Temp.	on healthy plant	Temp.	
Oct. 25	2 (n)	30 days	19–32°C	21–30 days	20–31°C	0
,,	1 (♂)	,,	,,	31–40	20–30	0
,,	2 (♀)	,,	,,	,,	,,	0
,,	1 (n)	,,	,,	,,	,,	0
,,	1 (♂)	,,	,,	41–50	,,	0
Aug. 25	3 (♂)	50	20–31	1–10	20–31	1 (Jan. 5)
,,	2 ,,	,,	,,	,,	,,	0
,,	2 (n)	,,	,,	,,	,,	0
,,	2 (♀)	,,	,,	11–20	20–30	0
,,	1 ,,	,,	,,	,,	,,	1 (Jan. 10)
,,	1 (n)	,,	,,	,,	,,	0
,,	1 (♂)	,,	,,	31–40	,,	0
Apr. 20'33	7 (♂)	30	20–38	11–20	13–34	0
,,	7 ,,	,,	,,	21–30	,,	0
,,	2 ,,	,,	,,	31–40	13–35	0
,,	1 ,,	,,	,,	41–50	15–36	0
May 20	5* ,,	5	20–36	11–20	12–34	0
,,	1* ,,	,,	,,	21–30	13–34	0
,,	7* ,,	,,	,,	31–40	13–35	1 (July 9)
,,	5* ,,	,,	,,	41–50	15–36	0
,,	1* ,,	,,	,,	51–60	16–36	1 (July 23)
,,	1* ,,	,,	,,	61–70	17–36	0
,,	5* ,,	10	18–35	11–20	12–34	0
,,	11* ,,	,,	,,	21–30	13–35	0
,,	1* ,,	,,	,,	31–40	14–35	0
,,	9* ,,	,,	,,	41–50	15–36	3 (July 17, 18, 19)
,,	3* ,,	,,	,,	61–70	18–37	0
June 6	4* ,,	50	18–38	11–20	22–37	0
,,	2* ,,	,,	,,	21–30	22–38	1 (Aug. 17)

* non-viruliferous progeny originated from an infective female leafhopper.

f) Results of experiments carried out in 1933

The leafhoppers used in this series of experiments were obtained from virus free colonies of leafhoppers which had been bred in the following manner. On April 14, thirteen leafhoppers were collected on *Astragalus sinicus* L. growing on rice fields at Shimadachi-mura near Matsumoto

and likewise 133 individuals at Shiraoka near Ômiya and in the vicinity of Utsunomiya the next day. Almost all the leafhoppers were in their nymphal stage but molted to become adults within a week. On April 17 and 18 the leafhoppers were confined singly on young healthy rice plants in separate glass tubes and allowed to remain there for a month. All but one individual proved to be free from virus and accordingly were transferred into insect proof cages enclosing healthy rice plants. The progeny of these insects were maintained on healthy rice plants to secnre virus free leafhoppers at the desired moment during the period of experiment. The non-viruliferous leafhoppers were allowed to feed upon diseased plants in an insect proof cage for a definite length of time ranging from 1 to 30 days and then confined singly to each healthy rice plant in a glass tube. They were transferred to new young plants at 10, 20, or 30 days' intervals. The resnlts of experiments will be shown below.

TABLE 22. Acquisition of infective power by the leafhopper (glass tube experiments in 1933)

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant		on healthy plant		
July 20	1 (n)	1 day	22–38°C	1–10 days	20–38°C	0
”	1 (♂)	”	”	11–20	21–38	0
”	1 („)	”	”	21–30	22–38	0
”	1 („)	”	”	31–40	21–37	0
”	1 (n)	”	”	”	”	0
”	5 (♂)	”	”	41–50	”	0
”	3 („)	”	”	51–60	”	0
”	1 (n)	”	”	”	”	0
”	1 („)	”	”	61–70	20–36	0
”	3 (♂)	”	”	71–80	19–36	0
”	2 (n)	”	”	”	”	0
31	6 („)	”	22–34	1–10	22–40	0
”	2 (♂)	”	”	11–20	22–39	0
”	9 (n)	”	”	”	”	0
”	7 („)	”	”	21–30	22–38	0
”	5 (♂)	”	”	”	”	0
”	2 („)	”	”	31–40	”	0
”	6 (n)	”	”	”	”	0
”	1 (♂)	”	”	41–50	20–37	0

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant		on healthy plant		
July 31	5 (n)	1 day	22–34°C	41–50 days	20–37°C	0
,,	2 (,,)	,,	,,	51–60	18–37	0
,,	5 (,,)	,,	,,	61–70	,,	0
July 20	2 (♂)	3 days	22–43	1–10	20–37	0
,,	7 (n)	,,	,,	,,	,,	0
,,	1 (♂)	,,	,,	11–20	21–38	0
,,	6 (n)	,,	,,	,,	,,	0
,,	4 (♂)	,,	,,	21–30	,,	0
,,	2 (,,)	,,	,,	,,	,,	1 (Aug. 25)
,,	1 (,,)	,,	,,	31–40	21–37	0
,,	5 (,,)	,,	,,	41–50	21–38	0
,,	1 (n)	,,	,,	51–60	20–37	0
,,	1 (♂)	,,	,,	61–70	19–37	0
31	1 (,,)	,,	22–40	1–10	23–40	0
,,	5 (,,)	,,	,,	11–20	22–39	0
,,	2 (,,)	,,	,,	31–40	22–38	0
,,	3 (,,)	,,	,,	41–50	20–37	0
,,	1 (,,)	,,	,,	51–60	,,	0
,,	9 (,,)	,,	,,	61–70	18–36	0
Aug. 8	1 (n)	,,	22–37	1–10	22–39	0
,,	3 (♂)	,,	,,	11–20	21–38	0
,,	5 (n)	,,	,,	,,	,,	0
,,	1 (♂)	,,	,,	21–30	,,	0
,,	2 (n)	,,	,,	,,	,,	0
,,	1 (,,)	,,	,,	31–40	18–37	0
,,	1 (,,)	,,	,,	41–50	18–36	0
,,	2 (♂)	,,	,,	51–60	,,	0
,,	5 (n)	,,	,,	,,	,,	0
June 16	14 (,,)	5	15–37	21–30	20–38	0
,,	13 (,,)	,,	,,	31–40	,,	0
,,	5 (,,)	,,	,,	41–50	21–38	1 (Aug. 3)
,,	13 (,,)	,,	,,	51–60	,,	0
,,	3 (,,)	,,	,,	61–70	,,	0
,,	1 (,,)	,,	,,	71–80	,,	0
,,	1 (,,)	,,	,,	101–110	20–37	0
July 6	19 (,,)	,,	21–39	1–10	22–36	0
,,	4 (,,)	,,	,,	11–20	21–36	0
,,	1 (,,)	,,	,,	21–30	,,	0

Date	No. of insects tested	Length of feeding period				No. of infective insects
		on diseased plant		on healtly plant		
July 6	9 (n)	5 days	21-39°C	31-40	22-38°C	0
"	3 (")	"	"	41-50	"	0
"	4 (")	"	"	51-60	"	0
June 16	11 (")	10	15-38	11-20	20-38	0
"	13 (")	"	"	21-30	21-38	0
"	23 (")	"	"	31-40	"	0
"	1 (")	"	"	41-50	"	0
"	2 (")	"	"	51-60	"	0
"	4 (")	20	17-38	1-10	"	1 (July 16)
"	10 (")	"	"	11-20	"	2 (July 21, 23)
"	10 (")	"	"	21-30	"	0
"	5 (")	"	"	31-40	"	0
"	6 (")	"	"	41-50	"	0
"	3 (")	"	"	51-60	"	0
"	14 (")	30	18-38	1-10	22-38	2 (July 24)
"	7 (")	"	"	11-20	21-37	1 (July 27)
"	4 (")	"	"	21-30	22-38	0
"	5 (")	"	"	31-40	"	0

The results of these experiments will be shown summarized in the following table.

TABLE 23. Acquisition of the infective power by the leafhopper

	Duration of feeding period on dwarf diseased rice plants (in days)										
	1	3	5	7	10	15	20	30	50	70	Total
No. of leafhoppers tested	130	122	246	24	313	56	118	230	43	18	1300
No. of infective leafhoppers	0	1	3	0	7	0	3	4	7	0	25
Percent. of infective leafhoppers	0	0.8	1.2	0	2.2	0	2.5	1.7	16.3	0	2.8

As shown above only 25 individuals of 1300 leafhoppers became infective after they had been allowed to feed upon diseased rice plants during a period from 3 to 50 days. The shortest period necessary for a non-viruliferous leafhopper to feed on diseased plants to pick up the virus was 3 days but a larger proportion of leafhoppers acquired the

virus after 10–50 days' feeding. The length of the incubation period of the virus in the insect carrier was not definitely determined, but the data available at present indicate that it was 10–40 days in certain cases where a temperature from 11° to 38°C. predominated as shown below, while it was prolonged to over two months in another case at a temperature ranging from 14° to 32°C. as mentioned before.

Insect No.	Feeding period on diseased plant	Feeding period on healthy plant		
	May 20–25 (20–36°C) (5 days)	May 25–June 4 (11–34°C) (10 days)	June 4–29 (13–36°C) (25 days)	
E′ 2	—	—	+ (July 9)	
E′ 7	—	—	+ (July 23)	
	May 20–30 (20–36°C) (10 days)	May 30–June 9 (12–35°C) (10 days)	June 9–July 9 (16–38°C) (30 days)	July 9–16 (21–38°C) (7 days)
E′ 35	—	—	—	+ (July 18)
E′ 36	—	—	—	+ (July 19)
E′ 47	—	—	—	+ (July 17)
	June 16–21 (15–37°C) (5 days)	June 22–July 5 (17–36°C) (13 days)	July 5–Aug. 3 (21–38°C) (29 days)	
F 20	—	—	+ (Aug. 3)	
	July 20–23 (22–43°C) (3 days)	July 23–Aug. 12 (22–37°C) (20 days)	Aug. 12–22 (20–37°C) (10 days)	
F 245	—	—	+ (Aug. 25)	

At any rate it is evident, as a result of these experiments, that the virus is not transmitted mechanically from diseased plants to healthy ones by the agency of leafhoppers.

8. *Host range of dwarf disease of rice plant*

The symptoms of dwarf disease of rice plant closely resemble those of corn stripe disease in Cuba and likewise those of streak disease of maize and sugar cane in South Africa. The latter diseases are transmitted by the agency of the leafhoppers, *Peregrinus maidis* ASHM.[205] and *Cicadulina mbila* NAUDE,[208, 209, 210] respectively. According to STOREY (1925)[208, 209] various species of cultivated and wild grasses are subject to the attack of streak disease of maize. The writer, accordingly, attempted to transmit dwarf disease of rice plant to corn and several other grasses by the agency of the leafhopper, *Nephotettix apicalis* var.

cincticeps. All the inoculated plants were raised in 5-inch pots in insect proof cages and the young plants 2 to 10 days after germination were exposed to the infestation of leafhoppers in cages or glass tubes as described before. The cages employed were 30 cm. square and 60 cm. high. The potted young plants were placed in the cages and a definite number of leafhoppers were introduced therein. When the plants were unfavorite food plants for the leafhopper, certain individual leafhoppers would not migrate to the plants to feed upon them but remained on the wall of their cages eventually to be starved to death. In the later experiments, therefore, glass tubes were used instead of cages, allowing 3 or 4 plants to be grown in a glass tube closed at the upper end by a thin cotton cloth and a definite number of infective leafhoppers were transferred on to these plants. The leafhoppers were allowed to remain there until they all died or the symptom of the disease began to appear. The results of these experiments are shown in the following table.

TABLE 24. Results of attempted transmission of dwarf disease of rice plant to cultivated and wild grasses

Series 1. Cage experiment

Date	Name of plant	No. of insect per plant	Length of feeding period	No. of plants	
				inoculated	affected
	Zea mays L.				
July 9'28	Unnamed variety	2	5–12 days	28	0
June 20'30	Longfellow & Sapporo Hachigyo	3	7–14	10	0
	Setaria italica BEAUV.				
June 30'30	Yûshi	3	"	5	0
	Echinochloa Crus-galli BEAUV. subsp. *colona* var. *edulis* HONDA				
June 30'30	Shiro-sangoku & Shiratama	3	20	10	5
July 31'30	Shiro-sangoku	2	21	10	9
	Panicum miliaceum L.				
June 30'30	Wase	3	20	5	5
July 31'30	Wase	2	"	10	7
Jan. 23'33	Wase	1	22	10	6
	Hordeum sativum var. *vulgare* HACK. subvar. *coeleste* HACK.				
Feb. 2'33	Marumi	1	5	10	0
Oct. 3'33	*Poa pratensis* L.	2	30	20	6

Series 2. Glass tube experiment (1933)

Date	Name of plant	No. of insect per plant	Length of feeding period	No. of plants inoculated	No. of plants affected
	Zea mays L.		days		
Mar. 1	Sapporo Hachigyo	1	7–10	5	0
"	Longfellow	"	"	6	0
3	Longfellow	"	"	5	0
20	Sapporo Hachigyo	3	5–7	10	0
21	Sapporo Hachigyo	10	"	5	0
	Hordeum sativum JESS. var. *vulgare* HACK. subvar. *coeleste* HACK.				
June 14	Marumi	2	7–13	10	0
	Hordeum sativum JESS. var. *hexastichon* HACK.				
Mar. 11	Chevalier	1	7–10	10	0
June 14	Chevalier	2	7–15	5	0
Mar. 11	Golden Melon	1	7–10	10	0
June 14	Golden Melon	2	7–15	10	0
	Avena sativa L.				
Feb. 28	White Belgium	1	7–10	10	0
May 14	White Belgium	1	7–15	10	1 (Jun. 8)
Mar. 13	Race Horse	1	"	10	1 (Apr. 17)
May 14	Race Horse	1	5–7	10	2 (Jun. 2, 7)
	Secale cereale L.				
Mar. 16	Unnamed variety	1	7–10	10	3 (Apr. 17)
May 14	Unnamed variety	1	5–8	10	0
	Triticum vulgare VILL.				
Mar. 16	Sapporo Harukomugi	1	7–10	10	2 (Apr. 17)
May 25	Sapporo Harukomugi	3	7–20	3	0
June 7	Sapporo Harukomugi	2	"	10	0
Mar. 17	Martin Amber	1	7–10	7	0
May 25	Martin Amber	3	7–20	5	0
June 7	Martin Amber	2	"	10	0
	Setaria italica BEAUV.				
Mar. 16	Yûshi	1	5–10	10	0
Mar. 17	Hôten 20-go	1	5–15	10	0
	Andropogon Sorghum BROT. var. *vulgaris* HACK. subvar. *japonicus* HACK.				
Mar. 14	Jagan	1	7–10	10	0
May 15	Jagan	1	5–7	20	0

Date	Name of plant	No. of insect per plant	Length of feeding period	No. of plants	
				inoculated	affected
	Andropogon Sorghum Brot. var. *obovatus* Hack.				
Apr. 5	Kuromi-barabo	1	7–10	5	0
	Panicum miliaceum L.				
Mar. 11	Wase	1	10	7	7
Mar. 16	Manshû-shiro	1	„	2	2
Apr. 5	Manshû-shiro	1	12	4	4
	Echinochloa Crus-galli Beauv. subsp. *colona* var. *edulis* Honda				
Apr. 5	Shiro-sangoku	1	„	10	10
Apr. 25	*Alopecurus fulvus* L.	1	10–15	10	7
May 9	„	1	„	5	1

In the experiments performed in 1928 and 1930, it was not definitely known whether the leafhoppers used were all viruliferous, although they all had been reared on dwarf diseased rice plants. Consequently the results may be regarded as inconclusive when negative. All the leafhoppers used in the experiments carried out in 1930 were progeny from infective female leafhoppers which had been bred on diseased plants so it is highly probable that almost all of them were viruliferous.

As shown in Table 24, it has been conclusively proved that *Panicum miliaceum* L., *Echinochloa Crus-galli* Beauv. subsp. *colona* Honda var. *edulis* Honda, *Poa pratensis* L., and *Alopecurus fulvus* L. are subject to attack by the virus causing dwarf disease of rice plant. The symptoms on these plants closely resemble those on rice plant, being characterized by streak and spotting on leaves and stunting of the whole plant. (Pl. I Fig. 2, Pl. II Fig. 5, 6) These plants proved to be favorite food plants for the leafhopper under consideration.

Rye, wheat and oats are slightly susceptible to the disease, infection having been produced in only a small portion of the inoculated plants. This may be partly due to the fact that these plants are uncongenial food plants for the leafhoppers which could not survive on them for longer than two or three weeks at most. The manifestations of the disease on these plants are generally less conspicuous but the severely affected plants are much stunted and the yellowish white spots and streaks on the leaves are rather striking when viewed by transmitted

light. (Pl I Fig. 3, Pl. II Fig. 7)

The plants which gave negative results are corn, Italian millet, barley and sorghum, although some of them may in the future prove to be more or less susceptible, if the work is carried out on a large scale. These plants were apparently unfavorable as food plants for the leafhoppers. They could survive on them for less than 15 days and perished sooner or later.

In "Results of experiments with insect pests. Report 8"[192] published by the Shiga Agric. Exp. Station in 1908, it is briefly mentioned that dwarf disease of rice plant was successfully transferred to *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA but all the attempts to transmit it to *Panicum miliaceum* L. and *Setaria italica* BEAUV. failed. It appears that similar experiments were repeatedly conducted with invariably the same results during a period from 1904 to 1912 as briefly stated in that station's Special Bulletin No. 2[193] and likewise in Annual Reports for the years 1908 to 1912.[194]

It is worthy of note that *Alopecurus fulvus* L. is susceptible to dwarf disease of rice plant. Since this is a biennial wild grass abundantly growing on the rice fields throughout Japan, it is possible that the virus causing dwarf disease of rice plant may be carried over the winter in this wild grass.

9. *Overwintering of the virus of dwarf disease of rice plant*

There are two possible ways in which the virus causing dwarf disease of rice plant survives over the winter: either in its insect carrier or in the perennial wild hosts.

On April 13, 1932, as stated before, about two hundred leafhoppers were collected on *Astragalus sinicus* L. growing on the rice fields in the vicinity of Shiraoka near Omiya and two days later about half the number were divided into several groups of 20 to 30 individuals and placed in as many insect proof cages enclosing young healthy rice plants. Twenty-nine days later, in one of the cages where about 30 leafhoppers were confined on as many healthy rice plants, one plant began to show distinct symptoms of dwarf disease. Thus it became evident that some individual leafhoppers, though probably few in number, which had hibernated on *Astragalus sinicus* and likewise on some wild grasses, carried the virus causing the disease.

In 1933 similar experiments were carried on by means of the glass tube method before described. The leafhoppers used were collected on

Astragalus sinicus growing on rice fields at Shimadachi-mura near Matsumoto on April 14 and likewise at Shiraoka near Ômiya and in the vicinity of Utsunomiya on the next day. Nearly all the leafhoppers were in their nymphal stage but molted to become adults within a week. On April 17 and 18 the leafhoppers were confined singly in a glass tube enclosing a young healthy rice plant and allowed to remain there for a month with the exception of a few leafhoppers which died in two or three weeks. The results of the experiment are shown in the following table.

TABLE 25. Infectivity of leafhoppers overwintered on *Astragalus sinicus* L. (1933)

Source of insects	Date of transfer of the insect to rice plant	No. of insects tested	Sex of insect	No. of infective insect
Shimadachi-mura	Apr. 17	13	male 4 female 9	0 1 (May 10)
Shiraoka	Apr. 17-18	83	male 31 female 57	0 0
Utsunomiya	Apr. 18	45	male 14 female 27	0 0
		146		1

As shown above only one individual of 146 leafhoppers tested proved to be infective. This leafhopper was collected on *Astragalus sinicus* growing on rice fields at Shimadachi-mura where dwarf disease of rice plant has been prevalent to some extent year after year.

In 1934 similar experiments were repeated again with the leafhoppers collected at three different localities, i.e., Utsunomiya, Kônosu and Matsumoto. These leafhoppers were supplied to the writer through the courtesy of Messrs. T. WATANABE, S. KATO and K. KURIBAYASHI. Although 300 to 400 leafhoppers were supplied only a third of them were found alive when they were received, a majority of them having died during their transport. The results of the experiments are shown in the following table.

TABLE 26. Infectivity of leafhoppers overwintered on *Astragalus sinicus* L. (1934)

Locality	Date of transfer of the insect to rice plant	*No. of* insects tested	Sex of insect	*No. of* infective insect
Utsunomiya	May 4	79	male 37 *female* 42	0 1 (*May* 20)
Kônosu	May 17	12	female	0
Matsumoto	May 28	14	female	1 (June 15)
		105		2

Of 105 leafhoppers tested 2 individuals, as shown above, proved to be infective.

The percentage of plants infected in these experiments is not high but the resnlts are considered to be conclusive owing to the conditions under which the experiments were performed. The rice plants for experimental purposes were grown in insect proof cages and each leafhopper was allowed to feed npon one rice plant in a glass tube where there could not be any source of accidental infection at any time throughout the experiment.

The above results, however, do not necessarily indicate that the virus of dwarf disease of rice plant overwinters in the leafhopper, because the virus may survive over winter in its pereunial wild hosts and subsequently may be picked up by the leafhoppers which feed upon these plants. The leafhoppers overwinter feeding on *Astragalus sinicus* L., *Alopecurus fulvus* L. and other grasses which are grown on the rice fields during the winter. They are especially abundant on *Astragalus sinicus* which is raised as the winter crop on the rice fields for ploughing in as a green manure. So far as *Astragalus sinicus* is not the symptomless carrier of the virus of dwarf disease of rice plant, it cannot be the source of virus since this plant has never been found to show any manifestation of virus disease. *Alopecurus fulvus* L. was experimentally proved to be subject to dwarf disease of rice plant as mentioned before. It is possible, therefore, that the virus may be carried over the winter in this wild grass. However, there are no records to indicate the occurrence of symptoms similar to those of dwarf disease of rice plant on wild grasses and the writer has never met with even one suspected case. Accordingly it is not definitely known to what extent this grass may

play a rôle as the source of virus causing the dwarf disease of rice plant. Rye, wheat and oats are also susceptible to dwarf disease of rice plant under certain conditions. It appears, however, that these plants have no possible relation to the overwintering of the virus of this disease, because they are not favorable food plants for the leafhopper which transmits the virus and moreover rye and oats are not raised at all in the regions where dwarf disease of rice plant is prevalent.

In short the conclusion seems undoubtedly that the virus causing dwarf disease of rice plant in all probability survives over winter in its insect carrier unless the fact is definitely established that the virus actually overwinters in *Alopecurus fulvus* L. and other biennial or perennial wild grasses under natural conditions or that *Astragalus sinicus* L. harbours the virus during the winter without showing any manifestation of the disease.

VII. Discussion and Conclusion

1. *Diseases allied to dwarf disease of rice plant*

The symptoms of dwarf disease of rice plant, its infectiousness, the invisibility of the causative agent under the microscope, the intracellular bodies in association with the affected tissues, and the mode of transmission, when taken altogether into consideration, lead one to the belief that this disease undoubtedly belongs to the virus disease group. Generally speaking, the virus diseases of plants can be classified based upon the most striking symptoms, into mosaic, curly leaf, leaf roll, yellows, streak and rosette. Dwarf disease of rice plant is a streak with a rosette appearance.

The virus diseases of plants can be divided into three groups, if the mode of transmission is taken into consideration. The diseases of the first group can be transmitted by the organic union only, such as budding or grafting, peach yellows,* peach rosette, little peach and phony disease of peach being representatives. The second group includes the diseases which are transmitted principally by the agency of insect carriers as represented by dwarf disease of rice plant, stripe disease of rice plant, streak of maize and sugar cane, corn stripe disease,

* Quite recently KUNKEL (1933)[119] successfully transmitted peach yellows to young seedling peach trees by the leafhopper, *Macropsis trimaculata*. Accordingly this should be transferred to the second group.

aster yellows and likewise by rosette of peanuts. In this group the transmission may be effected by grafting as already verified by KUNKEL (1926)[111] and STOREY and BOTTOMLEY (1928)[215] in aster yellows and rosette of peanuts respectively, but there is no evidence that these diseases are communicated through the juice from the affected plants. To the third group belong the diseases which are transmitted through the juice from the diseased plants, a majority of mosaic diseases and various other virus diseases being included in this group. The transmission by grafting and by the agency of insect vectors are usual in the third group. Certain diseases of this group have been shown to be seed borne while some others are transmitted through the soil.

Taking the symptoms and the mode of transmission into consideration, one comes to the conclusion that dwarf disease of rice plant closely resembles streak disease of maize and sugar cane which is transmitted by the agency of the leafhopper, *Cicadulina mbila*. Streak disease affects sugar cane, maize, teosinte, oats, *Panicum miliaceum* L. and some other grasses in South Africa (STOREY 1925).[208, 209] Stripe disease of rice plant which is characterized by yellowish stripes on the leaf and stunting of the whole plant and is transmitted by the leafhopper, *Delphacodes striatellus* FALL., more or less resembles dwarf disease of rice plant but in some respects it differs from the latter as pointed out by KURIBAYASHI (1931).[115] Corn stripe disease in Cuba is transmitted, according to STAHL (1927),[205] by the leafhopper, *Peregrinus maidis* ASHM. and somewhat resembles the rice disease under consideration in the manifestation of the disease. The corn mosaic investigated by KUNKEL (1921)[105] in Hawaii is also transmitted by the just named leafhopper (KUNKEL 1922,[107] 1927[112]) and is more or less similar to the disease in question so far as the striping of the leaves and dwarfing of the plant are concerned. Whether the corn stripe disease in Cuba is identical with the corn mosaic in Hawaii is not fully known. Anyhow these diseases are most closely allied to dwarf disease of rice plant. Aster yellows and curly top of beets are also transmitted by the agency of specific leafhoppers but the manifestations of these diseases are somewhat different from those of dwarf disease of rice plant.

2. *The relationship between the leafhopper and dwarf disease of rice plant*

It appears that the causal relation of leafhoppers to dwarf disease of rice plant has been held under suspicion since the time of the earliest

record of this malady, as obviously shown in a brief article entitled "Dwarfed rice plant, its cause and control measures" which was published by the Department of Education in the Official Gazette of October 18, 1890 as mentioned before. In this article they stated: "Since leafhoppers are extremely abundant on rice plants in the regions under consideration (Prefecture of Kyôto), sucking plant juice badly to suppress the growth of rice plant, some bodies ascribe the dwarfed condition of rice plants to the infestation of leafhoppers."

According to ISHIKAWA (1928),[86] HASHIMOTO (1894–95) was the first to prove experimentally the causal relation of leafhoppers to dwarf disease of rice plant. It is, however, uncertain with what species of leafhopper he worked. TAKATA (1895–96)[219] asserts that a certain species of leafhopper named "mon-yokobai" is responsible for the disease. The leafhopper which he called "mon-yokobai" is apparently *Deltocephalus dorsalis* MOTSCH. as shown by his description accompanied by illustrations. This leafhopper, however, has no bearing upon the disease as reported by the Shiga Agricultural Experiment Station (1900).[192]

In "Results of experiments with insect pests. Report I"[192] published by the Shiga Agricultural Experiment Station in 1899, it is stated that dwarf disease was caused by the leafhoppers, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL., *Deltocephalus dorsalis* MOTSCH., *Thamnotettix tobae* MATS., *Cicadula fasciifrons* STAL., and likewise by *Zygina limbata* MATS. (?) while none of the leafhoppers, *Delphacodes striatellus* FALL., *Tettigonia viridis* L. and *Nisia atrovenosa* LETH. was responsible for the incidence of the disease. It is evident that the disease was at that time entirely attributed to the infestation of leafhoppers. In Report II (1900) it is reported that only one species of all leafhoppers tested, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL. was capable of producing the true dwarf disease of rice plant, which was confirmed subsequently by long continued experiments. In Report VI issued in 1906, it is reported that dwarf disease was induced by the leafhoppers taken from Shiga Prefecture while those from Tôkyo failed to produce infections in healthy plants. This finding was confirmed in Reports VII and VIII published in 1908 showing that the leafhoppers taken from Okayama Prefecture produced the disease as well as those from Shiga Prefecture but those from Tôkyo and the northern part of Honshû (Main Island) were entirely uninfective. Furthermore it is stated that even the uninfective leafhoppers taken from Tôkyo or the

northern districts of Honshû acquired the ability to induce the disease when they were allowed to feed upon the dwarf diseased rice plants for about 5 days. Thus it became evident that the leafhopper itself does not cause the disease but transmits it acting as the vector. This was first shown by the experiments conducted in 1906 and confirmed during the subsequent years. Accordingly, it is in 1908 that the leafhopper, *Nephotettix apicalis* var. *cincticeps* was first authentically recorded to transmit dwarf disease of rice plant, although the causal relation of the leafhopper to the disease was proved 8 years before. Prior to this, according to BOKURA (1930)[21] and MURATA (1931),[149] Onuki, formerly entomologist of the Imperial Agricultural Experiment Station, succeeded in proving in 1902 (?) that the leafhopper, *Nephotettix apicalis* var. *cincticeps* acts as the carrier to transmit dwarf disease of rice plant. Onuki, however, published no account of it. ANDÔ (1910)[7] briefly reported the results of transmission experiments with the leafhopper carried out in the Imp. Agr. Exp. Sta. In 1905, according to him, the leafhoppers from Tôkyo were caged on dwarf diseased rice plants taken from Shiga Prefecture and the insects of the 2nd or 3rd generation proved to be capable of producing infections. He further stated that uninfective leafhoppers from Tokyô became infective when allowed to feed on diseased rice plants for 15 days (experiments carried out in 1906 and subsequent years).

As far as the writer is aware, curly top of beets is the first virus disease of plant in the foreign literature, shown to be transmitted by an insect. In 1906[11] BALL reported a great outbreak of the beet leafhopper, *Eutettix tenella* BAK. which seriously damaged the fields of Utah in the previous year and stated that "its punctures seem to cause a sort of thickening of the veins of the leaf and an unhealthy condition called 'curly-leaf' or 'blight'." In two succeeding papers (1907,[12] 1909[13]) BALL emphasized the causal relation between the leafhopper attack and the curly leaf and stated that the curly leaf condition apparently spread from leaf to leaf until finally the whole plant was affected, even though the leafhopper might have disappeared in the meantime. He (1909)[13] briefly referred to the experimental production of "curly-leaf" in cages arranged jointly by himself and E. G. TITUS. BALL's findings with regard to the causal relation of the beet leafhopper to curly top were confirmed by SHAW (1910)[189] and later by SMITH and BONCQUET (1915).[202] In 1915[22] BONCQUET and HARTUNG reported that the beet leafhoppers taken from wild plants had no power to

produce curly top but they became viruliferous after feeding for a few days upon affected plants. This fact was corroborated by SMITH and BONCQUET[202] in the same year.

A few years earlier than this, ALLARD (1912,[1] 1914[2]) asserted that the occurrence of mosaic disease of tobacco was associated with aphid infestation. According to him (1914,[2] 1917[6]) *Rhopalosiphum* (=*Myzus*) *persicae* SULZ.* may become an active carrier of infection in the green house, after it has been feeding upon mosaic diseased tobacco plants, while *Macrosiphum tabaci* PERG. appears to be an efficient carrier of the virus under field conditions.

Following these pioneer works many investigators have reported on various cases of insect transmission of virus diseases of plants. A relatively small number of them have been shown to be transmitted by leafhoppers; e.g., it has been reported that *Peregrinus maidis* ASHM. acts as the vector of the mosaic disease of corn in Hawaii (KUNKEL 1922)[107] and the corn stripe disease in Cuba (STAHL 1927),[205] that *Typhlocyba ulmi* L. spreads leafroll of potato (MURPHY 1923),[151] that *Cicadulina mbila* (NAUDE) and *C. zeae* transmit the streak disease of sugar cane and corn (STOREY 1925,[208, 209] 1931[213]), that *Cicadula sexnotata* FALL. disseminates aster yellows (KUNKEL 1924,[108] 1926[111]), that *Euscelis striatulus* acts as the carrier of the virus of cranberry false blossom disease (DOBROSCKY 1929,[47] 1931;[48] BECKWIRTH & HULTON 1929[16]) and that *Delphacodes striatellus* FALL. transmits the stripe disease of rice plant (KURIBAYASHI 1931).[115] Quite recently it has been shown that peach yellows is also transmitted by the leafhopper, *Macropsis trimaculata* (FITCH). (KUNKEL 1933)[113].

The relation of leafhopper to virus has been closely investigated in curly top of beet, streak disease of corn and aster yellows.

In curly top of beet SMITH and BONCQUET (1915)[202] demonstrated that non-viruliferous leafhoppers became highly infective after feeding upon diseased sugar beets for a period which at most needed not to exceed 3 hours. But the insects were unable to transmit the disease immediately after feeding on diseased plants and a period of at least 24 hours, but certainly no more than 48 hours, had to elapse before these insects could produce the disease. They came, therefore, to the conclusion that "the beet leafhopper does not mechanically transfer

* According to HOGGAN (1929),[79] this species of aphid is not capable of transmitting tobacco mosaic.

the virus from diseased to healthy plants but that some development or change takes place in the virus within the body of the insect during the first few hours after it feeds upon the diseased plant." They were the first to suppose the possible existence of the incubation period of virus within the body of the insect carrier. According to them, as short a period as 5 minutes is sufficient for a single leafhopper to feed upon a healthy sugar beet in order to produce infection. SEVERIN (1921)[186] states that the beet leafhopper can become infective by feeding only 1 or 2 minutes on a diseased beet and the minimum incubation period *of the virus in the leafhopper requires 4 hours at the temperatures:* maximum 103°F.; minimum 94°F. and mean 100°F. He later (1924)[187] reports that infective beet leafhoppers retained their infectivity during all of the nymphal stages, after each molt, and during the entire adult life and (1931)[188] that the shortest period necessary for a single leafhopper to transmit the disease was 7 hours. STAHL and CARSNER (1918)[206] report that newly emerged beet leafhoppers lifted from the plant before feeding were non-viruliferous but became carriers of the virus when fed on diseased heets. In their subseqnent papers (1923, 1924)[207, 34] they state that the shortest period required for non-viruliferous leafhoppers to feed upon diseased beets in order to obtain the virus was 10 *minutes, that the insects became infective within a period* of 15 hours after they had fed upon diseased beets and that the minimum period necessary for a single leafhopper to transmit the disease was 20 minntes for sugar beets and 10 minutes for *Stellaria media*. They further state that "a viruliferous leafhopper may not produce infection each time it feeds on a helathy plant, even though the periods of feeding be 24 or 48 hours or even longer" and that the ability to produce the disease is not inherent in the leafhopper. They pointed ont the apparent inability of any insects other than *Eutettix tenella* to transmit the disease.

The virus of aster yellows has been shown to require a much longer incubation period in its insect carrier, *Cicadula sexnotata* FALL. According to KUNKEL (1926),[111] a period of at least 10 days, generally 12 to 17 days must elapse before the leafhoppers become infective, after they have fed on yellowed aster plants. A feeding period of 2 hours upon the yellowed plant is not long enough to render the leafhoppers viruliferous but an exposure of one day is sufficient to enable them to become virus-bearing. Considerable variation is shown in the certainty with which each infective leafhopper transmits the disease. In this

respect KUNKEL states: "Certain individuals transmitted the yellows to every plant on which they were confined for one day and some others transmitted it over a long period of time and with great certainty, whereas another group of insects did it with considerable irregularity failing to produce infection in many of the plants on which they were confined, and still another gronp transmitted yellows to a few plants and then apparently lost the virus." He further states that viruliferous leafhoppers retained the virus for as long a period as 75 days when they were confined on the rye plants that are immune to aster yellows, but that the virus is not transmitted through the eggs of the insect carrier. He attempted to transmit aster yellows by different species of leafhoppers and other insects and by the red mites but only negative results were obtained.

In streak disease of maize, STOREY (1928)[212] has shown that the leafhopper, *Cicadulina mbila* (NAUDE) may acquire the virus after feeding for one hour on a diseased plant but in lower proportion than is the case when the feeding period is prolonged to several days. But "even when the feeding period on a diseased plant extends through the whole course of development of the insect from the first instar to the adult stage, a proportion of the hoppers remain uninfective." These insects are incapable of acting as vectors of the virus. The character of 'activity' in the transmission of maize streak has been found (1931)[214] to behave in inheritance as dominant to that of 'inactivity' and to be sex-linked. According to STOREY, the cross, active male × inactive female gave F_1 progeny of inactive males and inactive females. In the F_2 generation, active and inactive insects developed in approximately equal numbers in each sex. The reciprocal cross, inactive male × active female, yielded an entirely active F_1 offspring, with segregation in the F_2 generation into active and inactive males in about equal numbers and only active females. The incubation period of the virus in the insect is variable but shortest at the higher temperatures. The minimum period observed was 6–12 hours at 30°C. Infective power is apparently not lost during the process of molting and it is retained during periods of feeding as long as 2 to 3 months upon immune plants. The virus, however, does not pass from parent to offspring through the eggs.

In curly top of beet and maize streak, it appears that the relation of the virus to its insect vector is not absolutely specific, because curly top has been shown to be transmissible by another leafhopper, *Agallia sticticollis* STAL. in Argentine (FAWCETT 1927)[62] and maize streak is

transmitted by *Cicadulina zeae* as well as by *C. mbila* (STOREY 1931).[213]

In dwarf disease of rice plant, the leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL. seems to be specific to the virus which is not transmitted by any other leafhoppers.

As a result of the writer's experiments evidence was obtained to indicate that all individuals of the leafhopper, *Nephotettix apicalis* var. *cincticeps* are not capable of acting as vectors of the virus of dwarf disease. Certain individuals of this leafhopper fail to transmit the disease even when they have been hatched and reared on diseased plants, although no morphological difference can be found between these leafhoppers and those which readily transmit the disease. A majority of the progeny from an infective female, which have been reared on diseased plants are capable of producing infections in healthy rice plants. Sometimes a period of 5 minutes was sufficient for a single leafhopper to feed npon a healthy plant to transmit the disease and a feeding period of 30 minutes was long enough to enable most of the infective leafhoppers tested to produce infections in healthy plants. Most of the leafhoppers retained their infective power as long as they lived and certain individuals produced infection in every healthy plant on which they were confined for 24 hours on consecutive days. In comparatively few instances the infectivity of a leafhopper was apparently reduced or lost even under favorable conditions. A viruliferous leafhopper may not produce infection in every plant on which it feeds, even though the periods of feeding may be 24 hours or longer. It seems, therefore, that certain viruliferous leafhoppers sometimes fail to produce infections in healthy plants, probably owing to the temporary exhaustion of the virus in the salivary glands or the anterior portion of the alimentary canal. Then the infections subsequently produced by the same individual must be attributed to the virus which had either multiplied in the salivary glands etc. or migrated from some other portion of the body to the salivary glands and the anterior part of the alimentary tract. This is an additional evidence to indicate that the leafhopper does not transfer the virus mechanically from a diseased to a healthy plant. The virus may be transmitted through the eggs of infective leafhoppers. The progeny from infective female leafhoppers are either viruliferous or free from the virus whereas those from the crosses between uninfective females and infective males are entirely non-viruliferous. It appears that all the ova in the ovaries of an infective leafhopper are not always affected by the virus. A period from one to 14

days must elapse before most of the infective nymphs become capable of producing infections in healthy plants although certain individuals may be infective immediately after emergence from eggs. This admits several interpretations: either (1) the newly emerged tiny nymphs may be able to transfer a dose of the virus insufficient to produce infection in a healthy plant, or (2) some developmental changes or multiplication of the virus may occur within the insect body before it is fully infective, or (3) the virus may migrate from the other parts of the insect body to the salivary glands and the anterior portion of the alimentary canal. Most of these infective nymphs retain their infectivity during all the nymphal stages and through the entire adult life, without access of the virus. Only a small proportion of non-viruliferous leafhoppers, both nymphs and adults, became infective after feeding on dwarf diseased rice plants under the writer's experimental conditions. The minimum period observed to be necessary for a non-viruliferous leafhopper to feed upon a diseased rice plant to pick up the virus was 3 days. But the leafhoppers acquired the virus more readily when they had been allowed to feed upon diseased plants for 10 to 50 days. Certain individuals of the leafhopper, however, remained uninfective even when the feeding period on diseased plants extended to 50 or 70 days. In the case of dwarf disease of rice plant, there also occurs an incubation period of the virus in the insect carrier, which could not be definitely determined but was apparently more than 10–40 days in certain cases where a temperature from 13° to 36°C. predominated while it was prolonged to more than two months in another case at a temperature ranging from 14° to 32°C. This furnishes further evidence to show that the virus is not mechanically transferred from diseased to healthy plants by the agency of leafhoppers but that some development or multiplication of the virus takes place within the body of the leafhopper before it becomes fully infective.

In short the conclusion seems to be justified that a relatively small proportion of the leafhoppers acquire the virus after feeding on dwarf diseased rice plants but the individuals which have once become infective generally retain the virus through thei entire life and certain infective females transmit the virus to their progeny through the eggs. Thus it is evident that the leafhopper does not transfer the virus mechanically from diseased to healthy plants but that some development or multiplication of the virus takes place within the body of the leafhopper.

3. *Etiology of dwarf disease of rice plant*

Various theories have been advanced in order to account for the cause of dwarf disease of rice plant. KERNER et al. (1890),[92] as mentioned before, ascribed this malady to the products of the reduction process which occurs in the soil. On account of the deficiency of oxygen in the soil, they assert, the sulphates contained in the soil, manure and likewise in the irrigation water are brought to reduction, producing iron sulphide which suppresses the growth of rice plants.

This hypothesis was disproved by TAKATA (1895–96),[219] who looked upon this trouble as caused by the infestation of leafhoppers. As a result of more extensive studies performed by the Shiga Agricultural Experiment Station (1899–1905) the causal relation of the leafhopper, *Nephotettix apicalis* var. *cincticeps* to dwarf disease of rice plant was definitely established. A few years later, however, it was found that the disease is not due to the infestation of leafhoppers itself but that it is caused by certain infective principles which are transmitted by the agency of leafhoppers. The nature of the causative agent still remained unknown.

In the Annual Report of the Imperial Agricultural Experiment Station for the years 1913–1915,[85] issued in 1917, it is briefly stated that the occurrence of certain protozoa was noticed in the tissues of diseased rice plant as well as in the blood of the leafhoppers. However, no detailed account has been given of these protozoa or of their relation to dwarf disease of rice plant.

DAIKUHARA (1904)[39] was the first to recognize this trouble as a disease allied to sereh of sugar cane, mosaic disease of tobacco and likewise to the stigmonose of carnations. On this point YONEMARU and ASO's view (1909)[234] was quite in agreement with DAIKUHARA's opinion. In recent years KASAI (1924)[89] looked upon this disease as "allied to the virus diseases" and HINO (1927)[76] also emphasized that this disease belongs to the mosaic disease group.

The symptoms of the disease, its infectiousness, the invisibility of the causative agent under the microscope, the intracellular bodies associated with the affected tissues, and the mode of transmission point to the fact that this disease is undoubtedly caused by a certain virus. Then what is the virus?

The properties of virus have been demonstrated in mosaic diseases of plants, particularly in those of tobacco, cucumber, potato, bean and

others. Undoubtedly, however, the best known and most thoroughly investigated virus is that of the mosaic disease of tobacco, and numerous hypotheses have been advanced as to its nature. As has been discussed by the writer (1929)[66)] before, some of these hypotheses have been nearly discarded, whereas three of them are considered to be more plausible, namely, the chlamydozoa hypothesis of PALM (1922),[161] HUNGER's (1905)[83)] phytotoxin and allied views pointing to a certain toxin or enzyme-like substance, and the ultramicroörganism theory sngested by ALLARD (1916)[5)] and advocated by D'HERELLE (1924).[40)] Although it cannot be denied that the virus gronp in all probability is not a homogeneous one, these three hypotheses may be adopted in considering the nature of the virus of dwarf disease of rice plant.

PALM (1922)[161)] found certain foreign corpuscles and extraordinarily small granules in the cells of the mosaic diseased tissue of tobacco plant. He considered that these minute granules "agree in every respect with" the chlamydozoa of PROWAZEK (1907)[167)] or organisms occupying an intermediate systematic position between protozoa and bacteria, that the foreign corpuscles are homologous with the GUARNIERI bodies associated with variola, and further that these chlamydozoa represent the causal agency of mosaic disease of tobacco. However, no convincing evidence has been obtained to prove that these minute granules actually do represent the organisms which cause the disease. GOLDSTEIN (1924)[72)] suggested that these foreign corpuscles may represent a living organism or perhaps an aggregate of living organisms. But it appears that certain pathologists incline to conisder them as the reaction products of the affected cells. (IWANOWSKI 1903,[89)] PALM 1922,[161)] F. F. SMITH 1926,[196)] HOGGAN 1927[78)]) As stated before, the occurrence of similar intracellular bodies has been demonstrated in several other virus diseases of plants and certain investigators looked upon them as the causal organisms. The general belief among plant pathologists, however, seems to be in favor of the view that these bodies are the reaction products of the host cells rather than the causative agents of the disease. The intracellular bodies which are found in the chlorotic tissues of leaves and likewise in the root and crown of dwarf diseased rice plant closely resemble those foreign corpuscles occurring in association with the mosaic disease of tobacco. These bodies cannot be considered as protozoans or other microörganisms because no indication of protoplasmic differentiation is shown in these bodies. Minute granules have been revealed scattering within these intracellular bodies. It was not, how-

ever, definitely known whether they represent the so-called chlamydozoa. They are not in all probability chondriosomes because no similar granules are found in the cytoplasm of the host cells and on the other hand these granules could not be demonstrated by means of the technique ordinarily recommended for the study of chondriosomes. The intracellular bodies are present also in the chlorotic tissues of leaves of wheat, rye, *Panicum miliaceum* L., and *Echinochloa Crus-galli* BEAUV. subsp. *colona* var. *edulis* HONDA and likewise in *Alopecurus fulvus* L. affected by the virus of dwarf disease of rice plant. In shape, dimension and structures, these intracellular bodies closely resemble those occurring in the affected tissues of rice plant. Accordingly it seemed desirable to determine whether similar inclusion bodies also occur in the viruliferous leafhopper. After a careful examination of the salivary glands, the alimentary tract, and the egg follicles in the ovarian tubules, and likewise in the mycetome and other organs of the viruliferous leafhoppers, the writer was unable to discover any inclusion bodies or any visual evidence of the presence of the virus.

Except the intracellular bodies, no microörganisms or like bodies have been found in constant association with dwarf disease of rice plant in spite of a careful examination of serial sections of all parts of affected rice plants. As a consequence it is considered that the causative agent may be of an ultramicroscopic dimension, either the so-called ultramicroörganism or some toxin-like substance.

As has been stated before, a period of 5 minutes was sufficient for a single leafhopper to feed upon a healthy plant in order to transmit the disease. The average weight of an infective male or female leafhopper being abont 2.3 or 3.7 milligrams respectively, the amount of virns which a single leafhopper injects into the plant tissue during a period of 5 minutes is considered to be infinitesmally small. This extremely small quantity of the virus was capable of producing infection in a healthy rice plant whose average weight varied from 100 to 250 milligrams and developed a typical case of dwarf disease. The virus eventually becomes distributed throughout all parts of an affected plant as is obviously shown by the fact that the virus is present in the leaf tissues and is picked up by the leafhopper which feeds upon the leaf and that it exists also in the root since all the new growths show more conspicuous symptoms of the disease when the aerial parts of affected plants are cut back. In the green house the virus is persitent for years in the roots of affected plants and numerous diminutive tillers develop

invariably producing more and more striking symptoms of the disease when the plants are repeatedly cut back. The virus undoubtedly resides in the newly developed leaves, since a healthy leafhopper is capable of taking up the virus after feeding upon these leaves. Accordingly it is evident that the extremely small amount of virus which has been inoculated into a rice plant by the agency of leafhopper persists in the root forever and it is partly distributed throughout new growths of the plant. Such a condition is perpetuated for years in the green house and subsequently new growths invariably develop more and more conspicuous symptoms of the disease. This can be hardly explained, unless we assume that the virus multiplies in the plant body.

However, it is not certain whether the virus reproduces itself or the host cell affected with the virus produces the virus in turn,—that is some abnormal metabolic product of the affected cell may function as the virus and stimulate it to develop the same product. In this connection, it is interesting to refer to BAUR's view (1904)[14] as to the nature of viruses which cause the so-called infectious chloroses of plants or the variegation which is transmissible by means of grafting with infected twigs or even with single diseased leaves. He considered that the virus of the infectious chlorosis must multiply within the affected plant because, "durch Transplantation eines einzigen kranken Blattes könuen wir eine andere Pflanze infizieren, diese produziert eine unbegrenzte Menge kranker Blätter, mit jedem einzelnen von diesen Blättern können wir wieder eine andere Pflanze infizieren usf. ad infinitum." The virus, he argued, can not be an organism, since the disease can be transmitted by grafting only but not through the expressed juice of diseased leaves, nor by intimate contact with affected plants and otherwise, and since "ein parasitärer Organismus, der eine derartig geringe Fähigkeit hat, von einer Wirtpflanze in eine andere zu gelangen, ist überhaupt nicht existenzfähig." He suggested that the virus need not "aktiv wachsen" as an ordinary organism does, but that possibly some metabolic product of an affected plant may function as the virus and exert a formative stimulus on the embryonic leaf primordium, so that variegated leaves are produced instead of normal green foliage. Then these leaves may produce in turn the same abnormal metabolic product, which again exerts a stimulating action on the primordium of leaf. He looked upon certain infectious diseases, notably the mosaic disease of tobacco, as caused by such "ein stoffliches Virus."

HUNGER (1905),[83] evidently in ignorance of BAUR's paper, ad-

vanced a similar hypothesis as to the nature of the virus of mosaic disease of tobacco. He considered that that virus is a toxin or phytotoxin which is primarily produced as some metabolic product of plant cells and stimulates the normal cells to produce the same toxin.

BAUR (1906)[15] later inclined to consider it more probable that the virus is a highly organized chemical substance which is produced as *some metabolic product of the diseased plant and endowed with the* ability of growth, splitting other compounds to produce the same substance or synthesizing it. This substance acts on certain groups of molecules in the embryonic cells of leaves in the same way as the toxin does which was postulated by EHRLICH in his widely known side-chain theory. The newly produced toxin migrates throughout the plant tissues to reach the cell wherein it finds the free side chain with which to unite, thus coming to enter the embryonic cells of leaves.

BEIJERINCK (1898)[17] considered that the virus of tobacco mosaic is a contagium *vivum* fluidum, because it was possible, by starting with an extremely small quantity of the virus, to infect serially an endless number of plants and accordingly the virus must multiply within the living plant. ALLARD (1916)[5] also considered that the virus of tobacco mosaic was capable of increasing indefinitely within affected plants, and therefore "there was every reason to believe that it is an ultramicroscopic parasite of some kind."

BAUR and HUNGER, as stated before, also admitted that the virus *multiplies within the affected plant; nevertheless their views are opposite* to the considerations of BEIJERINCK and ALLARD that the virus is after *all, of a living nature.*

Now leaving as they are, the conflicting views whether the viruses of tobacco mosaic and infectious chloroses of plants are animate or inanimate, it should be desirable to return to the subject in question.

As stated before, some individuals of the progeny from the viruliferous female leafhopper carry the virus by nature and retain it during all the nymphal stages and through the entire adult life or for a period ranging from 53 to 97 days, without access of the virus. Furthermore, certain leafhoppers produced infections in almost every healthy plant on which they were singly confined for 24 hours when each leafhopper was transferred daily to a new healthy rice plant. The average weight of a nymph just emerged from the egg being 0.06 milligrams, the amount of virus contained in the insect body must be extremely small as compared with the inoculated plant which ranged from 150 to 300 milli-

grams by weight. Without assuming that the virus multiplies within the insect body, it can be hardly explained how such an insect is capable of producing infections in 35 to 71 healthy plants on which it was confined for 24 hours on consecutive days.

A period of more than 10 days must elapse before certain viruliferous nymphs become capable of producing infections in healthy plants whereas some other individuals may be infective immediately or a few days after their emergence from eggs. In such a case it is most reasonable to consider that the virus may multiply within the body of insect before it is fully infective as has been already pointed out.

Non-viruliferous leafhoppers are not capable of transmitting the disease immediately after they have fed upon diseased rice plants but a period of 10 to 40 days must elapse before these leafhoppers become infective. Under certain conditions the virus requires a much longer incubation period within the insect body. This seems to indicate that the virus must either multiply in the insect or undergo a definite change there.

Certain viruliferous leafhoppers, either nymphs or adults, occasionally fail to produce infections in healthy plants. It is in all probability due to the temporary decrease or exhaustion of the virus in the salivary glands and like organs. Then the infections subsequently produced by the same individual must be attributed to the virus which has multiplied in the salivary glands etc.

All the evidence available at present, therefore, seems to indicate that the virus of dwarf disease of rice plant multiplies within the body of the leafhopper.

Hereupon the question again may be advanced whether the virus reproduces itself or the host cell affected by the virus produces it in turn as some metabolic product of cells. If we assume the latter view it is almost impossible to elucidate why comparatively few individuals of the leafhopper which have fed upon diseased plants are capable of producing the virus and why a period of as long as one or two months must elapse before the leafhopper can produce the virus as the metabolic product of cells. How can the rice plant be affected by the metabolic product of the leafhopper, an exceedingly small quantity of which is injected through the proboscis into the plant tissues? Is it a toxin? If that be granted, is it then readily acceptable that the affected cells of the rice plant produce the same toxin? On the other hand, the concept cannot be admitted that the toxin produced by the affected cells of the

rice plant may be different from what is injected by the leafhopper, because it is almost impossible to conceive that both the toxin injected into the plant tissues and the toxin which is subsequently produced in the cells and different from the originally injected toxin stimulate the cells to liberate one and the same toxin as the metabolic product. If we assume that the virus of dwarf disease of rice plant reproduces iteslf within the insect body, it cannot be acceptable to suppose that the same virus is produced by the rice plant as a product of cell metabolism. Thus the evidence seems to lend support to the view that the virus reproduces itself both within the insect body and the rice plant.

Consequently it cannot be denied that the virus is autonomous and multiplies utilizing the substances found in its environment, transforming them into its own substance. Furthermore the virus has the power of adaptation to reproduce itself both within the insect body and the plant tissues. D'HERELLE (1924, 1926)[40, 41] asserts that the power of assimilation and the power of adaptation are two fundamental properties of life. On the other hand, MCKINLEY (1929)[131] points out that reproduction is perhaps the most fundamental of all functions of organisms, because life would cease to exist after a time if there were no reproduction and that adaptation is "closely following in importance the power of reproduction," since living beings would die if they did not possess the power of adapting themselves to their environment. According to these concepts it is inevitable to conclude that the virus of dwarf disease of rice plant is of living nature. The virus, as stated before, in all probability lies beyond the limits of microscopic visibility. However, nobody can deny with certainty the existence of organized beings of ultramicroscopic dimensions, since the limits of microscopic visibility cannot be the line of demarcation between living and nonliving beings.

Briefly stated, the sole hypothesis which is not in contradiction with any of the experimental results and which provides rational elucidation of all these facts is that the virus of dwarf disease of rice plant is a living entity of ultramicroscopic dimensions.

VIII. Summary

1. In the present paper a detailed account of dwarf disease of rice plant is given, presenting descriptions of the intracellular bodies associated with it, the results of transmission experiments and discus-

sions on the etiology of the disease.

2. The symptoms of the disease, its infectiousness, the invisibility of the causative agent under the microscope, the intracellular bodies associated with the affected tissues and the mode of transmission are considered to indicate that this disease belongs undoubtedly to the virus disease group.

3. Certain intracellular bodies are invariably present in the chlorotic tissue of the leaf as well as in the crown and root of the rice plant affected with dwarf disease. Similar bodies are found in the chlorotic tissues of the leaves of wheat, rye, *Panicum miliaceum* L., *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA, and *Alopecurus fulvus* L. affected with the virus of dwarf disease of rice plant. These bodies occur near or in close contact with the host cell-nucleus. They are usually round to oval but occasionally irregular in shape aud much larger than the host nucleus. They are of rather homogeneous structure containing many vacuoles of various size and resemble the intracellnlar bodies in association with certain virus diseases of plants and animals. All attempts to demonstrate either a nucleus or chromatin granules in these bodies were unsuccessful but minute granules were successfully revealed in these structures, the exact nature of which remains unknown.

4. Any inclusion bodies or microörganisms of etiological significance, or visual evidence of the presence of the virus could not be discovered in the salivary glands and the alimentary canal, egg follicles in the ovarian tubules, nor likewise in the mycetome and other organs of the viruliferous leafhoppers, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL., the insect vector of the disorder.

5. The disease is not transmitted through the soil or the seeds of rice plant. All attempts to transmit the disease by mechanical inoculations with unfiltered juice of the diseased plants or by means of leaf mutilation have failed. Artificial inoculation of healthy rice plants with the macerated tissues and body fluid of viruliferous leafhoppers yielded also only negative results.

6. The leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL. is the sole means of transmission of the disease, acting as the vector. However, all individuals of this leafhopper are not capable of acting as carriers of the virus. Certain individuals fail to transmit the disease even when they have been hatched and reared on diseased plants. A majority of the offspring from infective females, which have been reared

on diseased plants are capable of producing infections in healthy rice plants. Sometimes a feeding period of 5 minutes was sufficient for a single leafhopper to transmit the disease and a feeding period of 30 minutes was long enough to enable most of the infective leafhoppers tested to produce infections in healthy plants. Most of the infective leafhoppers retained their infective power as long as they lived and certain leafhoppers produced infections in every healthy plant on which an individual was confined for 24 hours on consecutive days. In comparatively few instances the infectivity of leafhopper was apparently reduced or lost even under favorable conditions. A viruliferous leafhopper may not produce infections in every plant on which it feeds, even though the period of feeding may be 24 hours or longer. It seems, therefore, that certain viruliferous leafhoppers sometimes fail to transmit the disease, probably owing to the temporary exhaustion of the virus in the salivary glands or the anterior portion of the alimentary canal. Then the infections subsequently produced by the same individual must be attributed to the virus which either multiplied in the salivary glands etc. or migrated from some other portions of the body to these organs.

The progeny from infective female leafhoppers are either viruliferous or free from the virus while those from the crosses between uninfective females and infective males are entirely non-viruliferous. It appears that the eggs are not affected by the virus after they have been deposited but they are probably attacked at an earlier stage of development in the ovaries of the maternal insect. All the ova in the ovaries of an infective leafhopper are not always affected by the virus. A period from 1 to 14 days must elapse before most of the viruliferous nymphs become infective although certain individuals may transmit the disease immediately after emergence from eggs. Most of these viruliferous nymphs retain their infectivity during all the nymphal stages and through their entire adult life, without access of the virus. Only a small proportion of nonviruliferous leafhoppers, both nymphs and adults, became infective after feeding on dwarf diseased rice plants. The minimum period observed to be necessary for a non-viruliferous leafhopper to feed upon a diseased rice plant to pick up the virus was 3 days but the leafhoppers acquired the virus more readily after 10 to 50 days' feeding. Certain individuals, however, remained uninfective even when the feeding period on diseased plants extended to 50 or 70 days. The incubation period of the virus within the inesct body could not be

definitely determined but it was apparently at least 10 to 40 days in certain cases where a temperature from 11° to 38°C. predominated while it was prolonged to more than 2 months in another case at a temperature ranging from 14° to 32°C. In short a relatively small portion of the nnmber of leafhoppers acquire the virus after feeding on dwarf diseased rice plants but the individuals which have ouce become infective generally retain the virus through their entire life and certain infective females transmit the virus to their offspring through the eggs. It is evident, therefore, that the leafhopper does not mechanically transfer the virus from diseased to healthy plants but that some development or multiplication of the virus takes place within the body of the leafhopper.

7. *Panicum miliaceum* L., *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA, *Poa pratensis* L. and *Alopecurus fulvus* L. are subject to attack by the virus causing dwarf disease of rice plant. The symptoms produced on these plants closely resemble those on rice plants. Rye, wheat and oats are slightly susceptible to the disease, infection having been produced in only a small portion of inoculated plants. This may be partly due to the fact that these plants are uncongenial food plants for the leafhopper. The plants which have given negative results are corn, Italian millet, barley and Sorghum. These plants were also apparently unfavorable as food plants for the leafhopper under consideration. Since *Alopecurus fulvus* L. is a biennial wild grass abundantly growing on the rice fields throughout Japan, it is possible that the virus may be carried over the winter in this wild grass. However, there is no record to show the occurrence of symptoms similar to those of dwarf disease of rice plant on wild grasses.

8. Certain leafhoppers which had been collected on *Astragalus sinicus* L. growing on rice fields in the spring proved to be viruliferous. Hence it is more probable that the virus survives over winter in its insect carrier unless it is definitely estahlished that the virus actually overwinters in *Alopecurus fulvus* and other biennial or perennial wild grasses under natural conditions or that *Astragalus sinicus* L. harbors the virus during the winter without showing any manifestations of disease.

9. All the evidence available at present seems to lend support to the view that the virus of dwarf disease of rice plant is autonomous and multiplies both in the insect body and in the plant tissues and that the virus in all probability lies beyond the limits of microscopic visibility.

The sole hypothesis, therefore, which is not in contradiction with any of the experimental results and which provides rational elucidation of all the facts is that the virus under consideration is a living entity of ultramicroscopic dimensions.

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EXPLANATION OF PLATE

Plate I.

Fig. 1. Healthy (left) and affected (right) rice plants.
Fig. 2. Healthy (right) and affected (left) plants of *Echinochloa Crus-galli* BEAUV. subsp. *colona* HONDA var. *edulis* HONDA.
Fig. 3. Healthy (right) and affected (left) wheat plants.

Plate II.

Fig. 4. Diseased leaves of the rice plant showing the typical streaks along the veins.
Fig. 5. Healthy (on the left end) and diseased leaves of *Echinochloa Crus-galli* subsp. *colona* var. *edulis*.
Fig. 6. Diseased leaves of *Panicum miliaceum* L.
Fig. 7. Diseased leaves of the wheat plant.

Plate III.

Fig. 8. A portion of a longitudinal section of the diseased leaf of rice plant, showing the intracellular bodies marked × (Fixed in GILSON's fluid and stained with GIEMSA's stains)
Fig. 9. do.
Fig. 10. A portion of a longitudinal section of the root of the affected rice plant, showing the intracellular bodies marked × (Fixed in chromo-acetic solution and stained with safranin and gentian violet)
Fig. 11. A portion of a cross section of diseased leaf showing the chlorotic tissue (Fixed in chromo-acetic solution and stained with safranin)
Fig. 12. A portion of the section shown in Fig. 8, highly magnified.
Fig. 13. A portion of the section shown in Fig. 9, highly magnified.

Plate IV.

Fig. 14. A portion of the section shown in Fig. 9, highly magnified.
Fig. 15 and 16. A portion of a cross section of the affected leaf of rice plant, showing an intracellular body in the mortor cell photographed at different optical levels to show the host cell-nucleus and the vacuoles in the body (Fixed in formol acetic alcohol and stained with safranin and gentian violet)
Fig. 17. A portion of a longitudinal section of a leaf of the wheat plant affected by the virus of dwarf disease of rice plant (Fixed in GILSON's fluid and stained with GIEMSA's stains) x: intracellular body, n: cell-nucleus.
Fig. 18. do.
Fig. 19. do.

Plate V.

Fig. 20. The leafhopper, *Nephotettix apicalis* MOTSCH. var. *cincticeps* UHL., male (left) and female (right)

Fig. 21. A longitudinal section of a male leafhopper (Fixed in GILSON's fluid and stained with MALLORY's stains)

Fig. 22. A longitudinal section of the head and prothorax of a male leafhopper, showing salivary glands (s), oesophagus (oe), cephalic ganglion (g) and muscels (m)

Fig. 23. A longitudinal section of the abdomen of a male leafhopper, showing the testes (t), mycetomes (myc), fat body (f) and midintestine (i)

Fig. 24. A longitudinal section of the head and prothorax of a male leafhopper, showing salivary glands (s)

Fig. 25. A longitudinal section of the abdomen of a male leafhopper showing testes (t) and midintestine (i)

Fig. 26. A longitudinal section of the abdomen of a male leafhopper showing the mycetome (myc), midintestine (i), fat bodies (f) and Malpighian tubules (M)

Plate VI.

Fig. 27. A portion of a longitudinal section of the abdomen of a female leafhopper showing the ovarian tubules (o), mycetomes (myc) and midintestine (i)

Fig. 28. A portion of the mycetome, showing the mycelium-like strands which fill up the mycetocytes.

Fig. 29. A portion of the mycetome. Note the inner mycetocytes which have collapsed, leaving the nuclei among clumps of cytoplasm.

Fig. 3

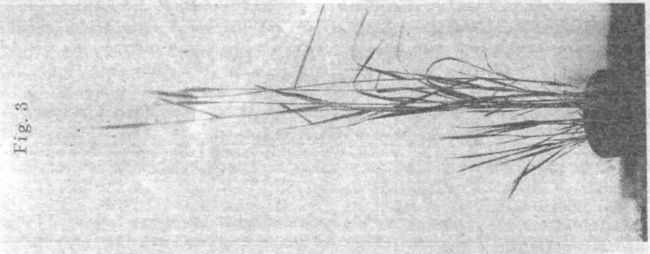


Fig. 2

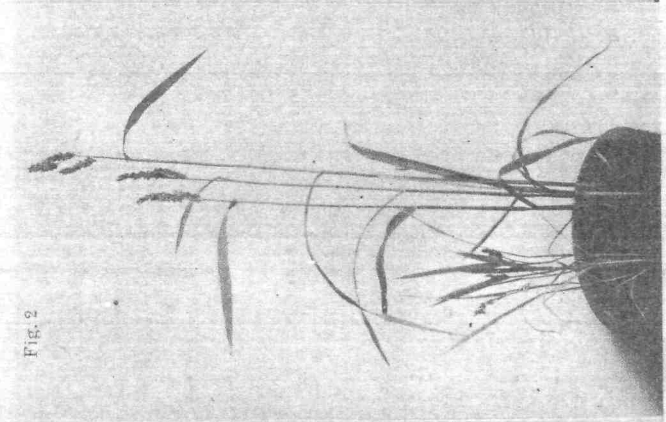


Fig. 1

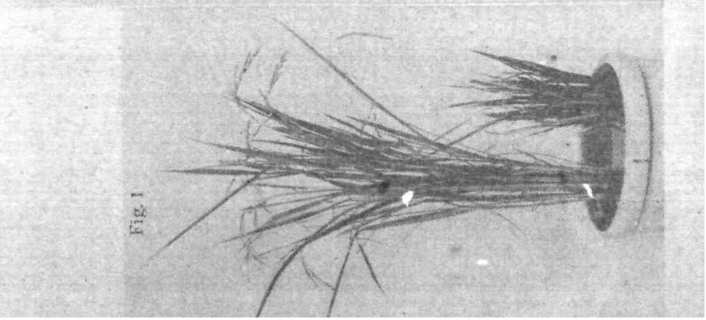


Fig. 3

Fig. 2

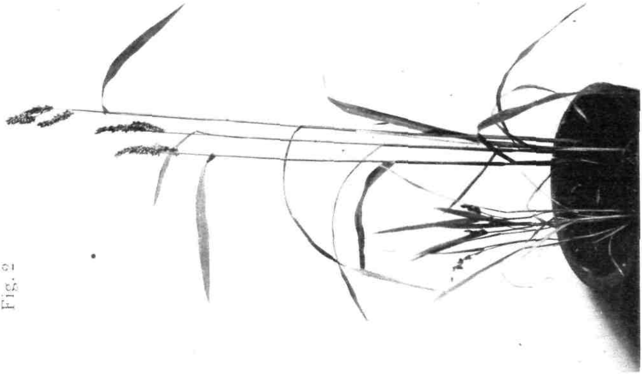


Fig. 1

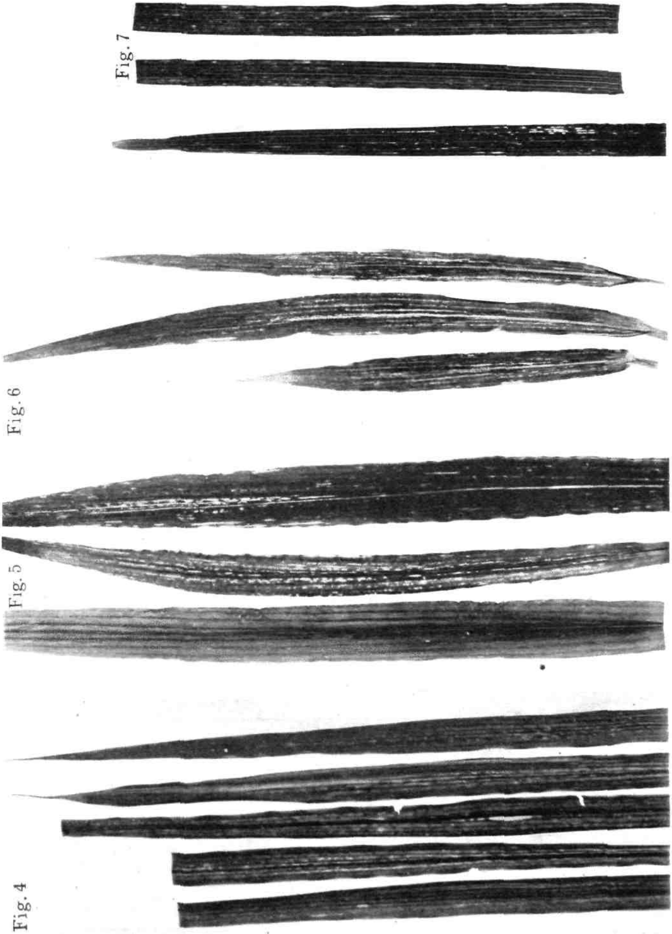


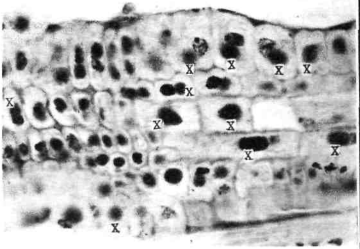
Fig. 7

Fig. 6

Fig. 5

Fig. 4

Fig. 8



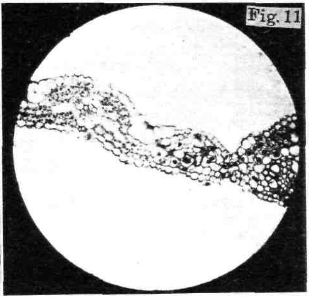
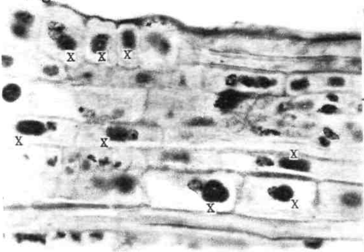


Fig. 11

Fig. 9



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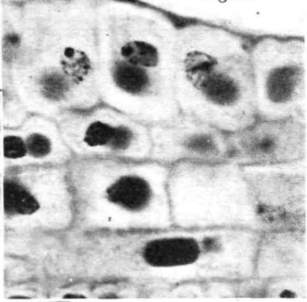


Fig. 10

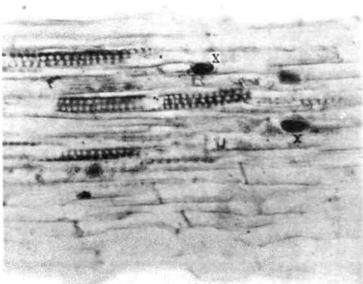


Fig. 13

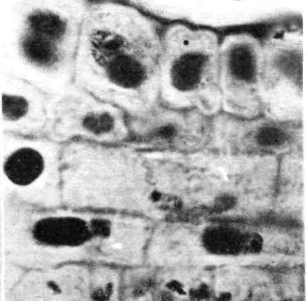


Fig. 14

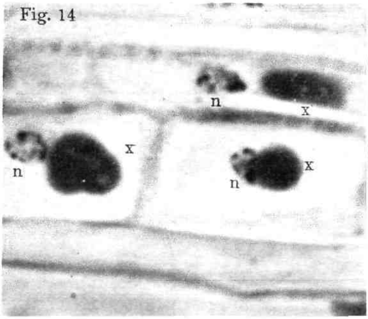


Fig. 15

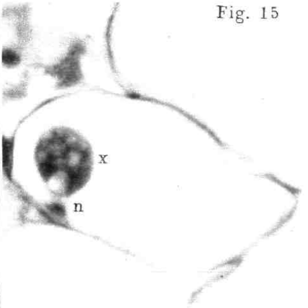


Fig. 17

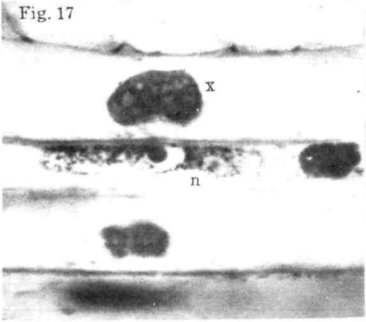


Fig. 16

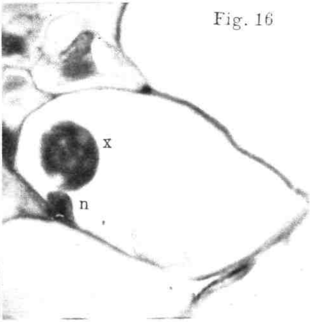


Fig. 18

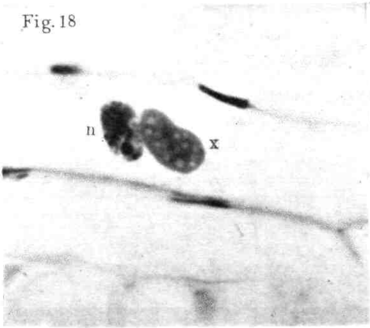
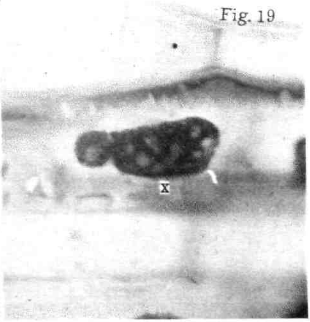


Fig. 19



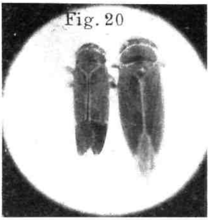


Fig. 20

Fig. 21

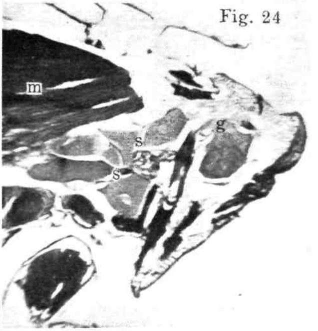


Fig. 24
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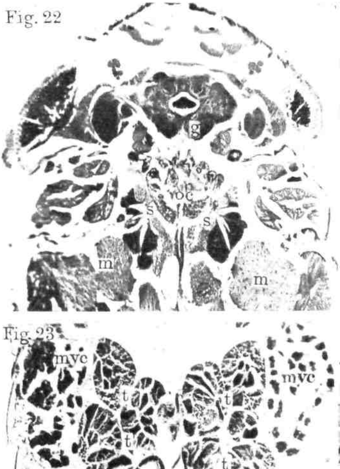
Fig. 22
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m

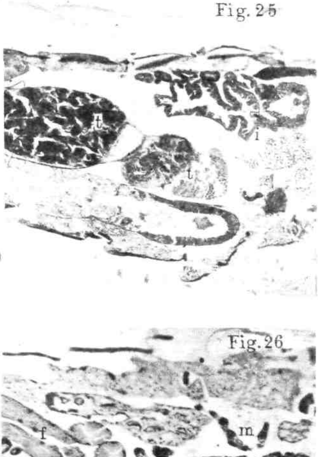
Fig. 25
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i

Fig. 23
myc
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Fig. 26
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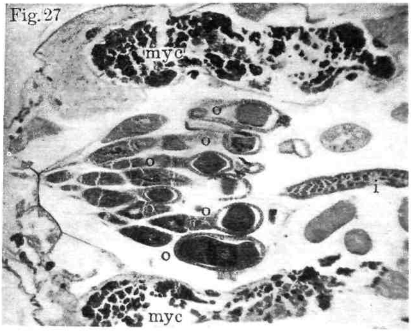
Fig. 27
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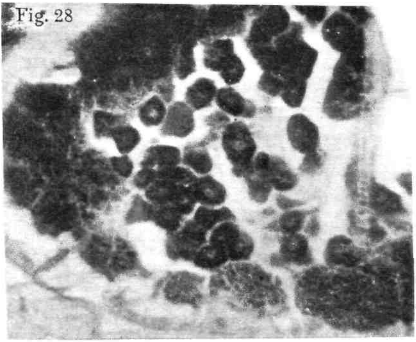
Fig. 28

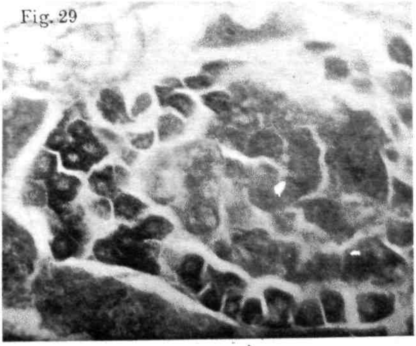
Fig. 29

CHEMICAL STUDIES ON *RHIZOPUS JAPONICUS*

By

Hoshik Lim

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INTRODUCTION

A most interesting piece of research is that on the utilization of microörganisms for nutrition. They can be cultured in quite confined spaces and they grow so rapidly that one may presume to employ them in artificial diets, particularly for some most important nutritive elements. From this standpoint, the author has made some chemical investigations on *Rhizopus japonicus*, hoping to gain some conception of its nutritive value.

Rhizopus japonicus is a saccharifying fungus, and was found by Boidin[1] in some Japanese "Koji". It was Vuillemin[2] who described it as a species of *Rhizopus*. Afterwards, numerous reports were published, but most of them are microbiological, and the chemical studies are very few: Cramer[3] Marschall[4] and others have reported on the ash and nitrogen contents of *Mucor* species: Nikolski[5], on the relation between the nitrogen content and the time of culture: Munz[6], Gerard[7] and Wisselingh[8], isolated trehalose, ergosterol and chitin respectively. Recently Nielsen[9] proved the existence of some materials which promote the growth of yeast and named them "Rhizopin". The author has studied the components such as the lipid, carbohydrates, proteins and vitamins of *Rhizopus japonicus*, from the view point of nutrition, the results of which studies will be described here.

CHAPTER I

PREPARATION OF SAMPLE

The species of fungus used in this experiment is *Rhizopus japonicus Vuillemin*, specimen kept in the Institute of Applied Mycology, College of Agriculture, Hokkaido Imperial University.

As a culture solution, Raulin's solution[10] was used of which the composition was as follows:—

1.	Tartaric Acid	2.667%
	and magnesium Carbonate	0.267 "
2.	Ammonium Nitrate	2.667 "
3.	Ammonium Phosphate	0.400 "
4.	Potassium Carbonate	0.400 "
5.	Ammonium Sulphate	0.167 "
6.	Zinc Sulphate	0.047 "
7.	Ferric Sulphate	0.047 "
8.	Sodium Silicate	0.047 "
9.	Thirty five grams sucrose, purified with alcohol, was dissolved in 150 cc. of distilled water.	

Twenty five cc. of each of 1, 2, 3, 4, 5, 6, 7 and 8 were mixed and to this mixture 50 cc. of the 9 solution was added.

As is well known, the culture medium has a great influence on the character of the fungus. Recently Yamamoto[11] studied the relation between the culture media, their temperature and the fermentation of the *Rhizopus* species.

The stock fungus used by the author was cultured in Koji agar. The organic substances in Koji agar would therefore probably influence the fungus. Attempts were made to eliminate these probable effects of the medium by successive transferrences into Raulin's solution for 20 generations. As Raulin's solution contains no organic substance whatever except sucrose and tartaric acid, any influence caused by the other organic substances was negatived.

The successive culturing was carried out every 2–3 days, and the fungus of the 20th generation was cultured once more in Raulin's solution as follows.

Two hundred and fifty cc. of Raulin's solution were transferred into 1-liter Erlenmeyer's flasks. Plugged with cotton, they were sterilized at 100°C. in Koch's sterilizer for three successive days, for 30 minutes

every day. The flasks were then placed in an incubator of 28°–30°C. overnight to ascertain their sterility; next inoculation was made with the least possible quantities from the above material and kept at 28°–30°C. for 5–7 days. Thus 0.5–1.2 grams fungous bodies were obtained per flask. By this method 1.5 kg. of the sample was prepared.

To separate the fungous bodies thus grown on the surface of the culture solution, the solution was decanted off, and, to wash off the culture solution completely, they were washed with distilled water several times. By means of a pincette the fungous bodies were taken out, pressed between filter papers, and dried in a sulphuric acid desiccator under negative pressure. When completely dried, they were crushed in a mortar, and kept in a bottle in a cool place.

To examine their general composition, 5 grams of the sample were treated successively with ether, 95% alcohol, and water: the soluble matters in each of these solvents were determined to be:

Ether soluble matters	9.720%
Ninty five percent alcohol soluble matters	4.635 "
Water soluble matters	23.133 "
Insoluble matters	62.512 "

The general analysis of the sample shows the following results.

TABLE I
Constituents of *Rhizopus japonicus*

	In air dried matter %	In dry matter %
Water	5.336	—
Crude protein	32.988	38.847
Crude fat	9.201	9.720
Crude fiber	7.285	7.696
Nitrogen free extracts	39.961	42.214
Ash	5.230	5.525
Total nitrogen	5.278	5.575
Non-protein nitrogen	3.431	3.624

Two hundred grams of the sample were ignited and ten grams of white coloured ash were obtained. Its composition was determined.

TABLE II
Mineral composition of *Rhizopus japonicus*

SiO_2	25.556%	P_2O_5	37.233%
Fe_2O_3	1.482 "	MgO	1.326 "
SO_3	0.314 "	K_2O	18.575 "
Na_2O	9.786 "	ZnO	0.128 "
The rest	5.600 "		

CHAPTER II

ETHER SOLUBLE MATTERS

As already shown, the ether soluble matters of the fungous bodies amount to about 9.72%. To study their character, from 1 kg. of the dried sample all ether soluble matters were extracted in the following way.

One kg. of dried sample was extracted with ether in Soxhlet's apparatus for a week; completely to get the still remaining ether soluble matter in the residue, it was treated with 95% alcohol in a cool place for 5 days. It was extracted further 3 times with hot 85% alcohol at 85°–90°C. for 10 hours. All these alcohol extracts were cooled to room temperature, all the precipitates which appeared by cooling filtered off; after the removal of alcohol by evaporation at low temperature the remainder was dissolved in water and the solution was repeatedly shaken with ether. This ether extract and the ether extract in the apparatus were mixed together. After dehydration by the addition of anhydrous sodium sulphate, the ether was evaporated. Thus 98 grams of brown solid fats were obtained.

When these fats were treated with acetone 1.5 grams of insoluble substance were precipitated.

(1) General characters of the fats

Through the fraction soluble in cool acetone, CO_2 gas was passed at a low temperature for quite a long time, and kept in a vacuum desiccator for a week. Thus when the acetone was completely removed, 96 grams of brown solid fats were obtained.

By ordinary analysis the following results were obtained for these fats.

Table III

General characters of the fats

Specific gravity (at 40°C.)	0.965
Refractive index (at 40°C.)	1.457
Acid value	95.40
Iodine value	84.65
Reichert-Meissl value	8.16
Acetyl value	38.00
Saponification value	164.00
Hehner value	72.75
Polenske value	0.82
Ester value	68.60
Unsaponifying matters	3.78%
Unsaturated fatty acids	61.25%
Saturated fatty acids	38.75%

From the above results, it may be seen that these fats resemble the general fats. However the acid value is comparatively higher than the saponification value and also the Reichert-Meissl value is high when compared to the fats.

(2) Unsaponifying matters

(a) *Isolation of ergosterol and biological test of it*

Seventy grams of these fats were saponified for an hour according to the Lewkowitsch method[12]. The seventy grams of fats were taken in a one liter flask, and, after the addition of 250 cc. alcohol, 35 grams potassium hydroxide and 140 cc. distilled water, they were saponified for an hour on a boiling water-bath. To the saponified solution, after the removal of alcohol by CO_2 gas admission, a large quantity of water was added and repeatedly shaken with ether. All these ether extracts were again saponified as stated, and, after the saponification was completed, again shaken with ether, and dehydrated with anhydrous sodium sulphate. The ether was then evaporated. After laying aside, lustrous, scale-like crystals were abundantly obtained with absolute alcohol and ether the yield being 3.48 grams.

The crystals thus obtained were positive for Liebermann and Salkowski's reaction, and precipitated by digitonin, characteristic of sterol. As Tanret[13] and Ellis[14] isolated fungisterol and ergosterol from fungous bodies, and Gerard[7], ergosterol from *Mucor mucedo*, the obtained substances were examined in this respect. Three and four-tenths grams of the crystals were dissolved in 20 cc. of anhydrous acetic acid, and small pieces of anhydrous sodium acetate were added. After 3 hours boiling, 150 cc. of hot water were poured in and stirred. The oily fraction was collected and dissolved in ether; the ether soluble fraction was then washed several times with water in a separatory funnel, dehydrated, and the obtained acetate, after the evaporation of ether, was treated with acetone according to Tanret's method[13]. The soluble part was removed, and by recrystallization with acetone 0.85 gram of scale-like crystals was yielded.

The melting point of the crystals was 168°–171°C., the refractive index and the elementary analysis were as follows.

Refractive index:

One per cent alcohol solution; tube length, 10 cm.; temperature, 17°C.; Ventzke sugar scale, −2.5; corrective coefficient, 0.3465.

$$[\alpha]_D^{17^\circ}=\frac{-2.5\times 0.3465\times 100}{1\times 1}=-86$$

Elementary analysis:

Sample	CO_2	H_2O	C	H
g.	g.	g.	%	%
5.824	17.53	5.51	82.09	10.51
5.645	16.99	5.33	82.08	10.49
$C_{30}H_{46}O_2$	—	—	82.07	10.37

From these data, the crystal will be seen to resemble ergosteryl-acetate. To verify this the crystalline substance was again saponified. and recrystallized with alcohol. Seventy eight-one hundredths gram acicular crystal was obtained.

The crystal thus obtained is positive for Liebermann and Salkowski's reaction; the melting point is 159°–162°C., and the refractive index is as follows;

One per cent chloroform solution; tube length, 10 cm.; temperature, 18°C.; Ventzke sugar scale, −3.5; corrective coefficient, 0.3465.

$$[\alpha]_D^{18^\circ}=\frac{-3.5\times 0.3465\times 100}{1\times 1}=-120$$

From the above data, the crystal is seen quite definitely to be ergosterol.

Ergosterol will be activated to vitamin D when irradiated by the ultra-violet ray, and even quite small quantities are capable of curing experimental rachitis in rats, as has already been proved by Windaus[(15)] and Rosenheim[(16)], and substantiated by many other investigators. With the obtained crystal, biological tests were therefore executed on albino rats.

Biological Test

As the basal diet of albino rats, Steenbock's No. 2965[(17)] was employed; when the rats are fed on the basal diet alone, characteristic skeletonial changes due to rachitis may be seen, owing to the shortage of vitamin D. Ossified tissue is formed at the basal end of the tibia by the lack of preeipitation of calcium salts; but when the D-factor is supplied to those suffering from rachitis, the disease will be completely cured within 3 weeks. Thus, noting the presence or the absence of this disease in those animals which were fed with basal diet only and in those which were fed with the test sample accompanying the basal diet

for 5 weeks one can know easily the vitamin D content of the test sample. For diagnosis the animals were examined with Röntgen-rays. It was also investigated if the tested sample would cure the attacked rats in 3 weeks or not. For the generation of ultra-violet rays, a Shimazu Mercury Lamp was used of 2 amp. at 90–100 volts. The sample dissolved in olive oil was irradiated for 40 minutes, at a distanee of 40 cm. from the source. The sample was given *per os*.

The resnlts thus obtained were as shown in Tables IV and V. Among the rats, No. 260, which was fed on the basal diet alone, No. 261, which took daily 1 cc. of irradiated olive oil in addition to the basal diet, and No. 263, for which 1 cc. of irradiated Raulin's solution was added to the hasal diet every day, suffered equally seriously. Among the other rats, No. 265, which was fed on the basal diet and on 0.1 cc. of irradiated cod-liver oil, and No. 266, which took 1/500 mg. of irradiated sample and the basal diet every day, kept their health quite well after 2 mouths feeding. (see Table IV).

Of the four rats typically attacked by the disease after 5 weeks feeding with basal diet alone, two rats, Nos. 267 and 268, received an addition of 0.2 cc. of irradiated cod-liver oil to their daily diet. To the diet of the other two, Nos. 260 and 261, 1/1000 mg. of irradiated sample were added every day; after 3 weeks feeding, it was seen that all these rats had been completely cured. (see Table V).

TABLE IV

Effect of the sample upon the rachitis of rats

Animal No.	*Duration of* experiment (days)		Body weight: Initial weight	Body weight: After disease	Röntgen diagnosis
260	35	Steenbock No. 2965.	28	68	Serious
261	"	Steenbock No. 2965 1 cc. of irradiated olive oil.	40	65	"
263	"	Steenbock No. 2965 1 cc. of irradiated Raulin's solution.	32	60	"
265	"	Steenbock No. 2965 0.1 cc. of irradiated codliver oil.	36	82	Ordinary
266	"	Steenbock No. 2965 1/500 mg. of irradiated sample.	41	95	"

TABLE V

Curative effect of the sample upon the rachitis of rats

Animal No.	Basal diet	Duration of expt. (days)	Sample	Duration of expt. (days)	Body weight			Röntgen diagnosis	
					Initial weight	After disease	After expt.	After disease	After expt.
					g.	g.	g.		
267	Steenbock No. 2965.	35	0.2 cc. of irradiated cod-liver oil.	21	42	86	105	Serious	Completly cured
268	"	"	"	"	36	60	82	"	"
260	"	"	1/1000 mg. of irradiated sample	"	38	68	93	"	"
261	"	"	"	"	40	65	85	"	"

From these results it may be inferred that the crystal is doubtlessly ergosterol.

(b) *Isolation of fungisterol*

When kept at room temperature, crystalline substances appeared in the filtrate after the separation of ergosterylacetate. The melting point of these crystals was 158°C., which agrees with that of fungisterylacetate. The crystals were saponified, and recrystallized in alcohol; 1.85 grams of crystal were thus obtained.

The melting point of this recrystallized substance is 143–145°C. The refractive index and the elementary analysis are as follows:

Refractive index

Three per cent chloroform solution; tube length, 10 cm.; temperature, 18°C.; Ventzke sugar scale, −1.9; corrective coefficient, 0.3465.

$$[\alpha]_D^{18^\circ} = \frac{-1.9 \times 0.3465 \times 100}{1 \times 3} = -23$$

Elementary analysis:

Sample	CO_2	H_2O	C	H
mg.	mg.	mg.	%	%
4.212	13.055	4.359	84.53	11.50
4.362	13.455	4.829	84.08	12.32
$C_{25}H_{40}O$	—	—	84.27	11.24

From the results obtained, it is to be judged that the crystal is apparently fungisterol.

(3) SAPONIFYING MATTERS

(a) *Isolation of saturated fatty acids*

The saponified solution, after the removal of excessive ether by

warming on the water-bath, was cooled, mixed with a large quantity of water, and neutralized with dilute acetic acid. Ten per cent lead acetate solution was added under gentle agitation. Fine precipitates appeared. After having been put away overnight, the clear supernatant liquid was filtered off and the precipitates were successively washed with water until they became completely free from lead, a fact which was ascertained by testing with sodium carbonate. The precipitates were then shaken with ether, and stood overnight. The material was then centrifuged to separate the precipitates from ether. This procedure was repeated, and the precipitates thus obtained were dissolved by the addition of dilute hydrochloric acid and shaken with ether. The ether solution was then washed with water until it became quite free from chloride. Dehydrated by the addition of anhydrous sodium sulphate, the ether was evaporated off. Thus 31.2 grams of yellow, solid fatty acid were obtained.

General characteristics

The melting point and neutralization value of this fatty acid were determined in the ordinary way, and the iodine value by Wij's method.

Melting point	56°–58°C.
Neutralization value	214.2
Iodine value	4.85

From the fact that the iodine value is 4.85, it is clear that the fatty acid certainly contains a small quantity of unsaturated fatty acids, but because the quantity is very small they were at once methylated according to Haller's method[18] without giving them much attention.

Fifteen grams of these saturated fatty acids were dissolved in 18.8 grams of anhydrous methyl alcohol and 26 grams of ether containing 2.5% hydrochloric acid, and heated for 12 hours with a reflex condenser. Cooled, the solution was neutralized with barium carbonate, and brought into a separatory funnel. Excessive methylalcohol was washed off with sodium chloride solution. The ethereal fraction was then dehydrated with anhydrous sodium sulphate and the ether was evaporated. Fourteen and four-tenths grams methylester of fatty acid were obtained.

The methylester thus obtained was distilled under a pressure of 12 mm. atm.; distillation began at 180°C. Fractional distillation was

therefore made at 180°–190°C., 190°–200°C. and 200°–210°C. The fatty acids thus obtained were as follows.

TABLE VI
Effects of fractional distillation of fatty acids at varying temperature

Distillation Number	Temperature C.	Atm. pressure mm.	Saponification value	Melting point C.	Yield g.
I	180–190	12	208.2	28	3.5
II	190–200	,,	205.7	29	6.2
III	200–210	,,	206.5	29	2.4

After this distillation 1.8 grams of dark brown residue were found in the flask.

From the above data, all methylesters of these fractions I, II and III seem to consist mostly of palmitic acid methylester. The three fractions were therefore mixed together, saponified with alcoholic potash solution, and then slightly acidified with hydrochloric acid. Free fatty acid was obtained. It was recrystallized in alcohol, the melting point of which was 60°C. After several recrystallizations in alcohol, 5.3 grams of white acicular crystal were obtained.

The melting point of this recrystallized white sample is 62°C. and the neutralization value 218.8 both of which figures agree with those of palmitic acid. Elementary analysis shows the following results.

Sample g.	CO_2 g.	H_2O g.	C %	H %
0.0562	0.1543	0.0638	74.88	12.61
$C_{16}H_{32}O_2$	—	—	74.93	12.58

Therefore, the crystal is certainly palmitic acid.

The dark brown mass remaining in the distillation flask was saponified with alcoholic potash solution, and then acidified slightly with hydrochloric acid solution. The fatty acid thus freed was washed with ether in the separatory funnel. This ether solution was repeatedly washed with water to remove the hydrochloric acid, then dehydrated with anhydrous sodium sulphate; ether was evaporated off and fatty acid was obtained. On fractional crystallization, fatty acids with melting points of 68°C. and of 28°C. were obtained. When both these crystalline substances were recrystallized with alcohol, the former was obtained as a lustrous laminar crystal, and the latter as a white acicular

one. The melting points were 69°C. and 61.5°C. respectively, and the yields were 0.28 gram and 0.85 gram. The neutralization values were 197.88 and 216.85 respectively.

	Yield	Melting point	Neutralization value
Laminar crystal	0.28 g.	69°C.	197.88
Acicular crystal	0.85 g.	61.5°C.	216.85

From these results it is clear that the acicular crystal must be palmitic acid and the laminar crystal stearic acid. The latter was subjected to elementary analysis, the results of which were as follows;

Sample g.	CO_2 g.	H_2O g.	C %	H %
0.1254	0.3468	0.1447	75.42	12.83
0.0883	0.2449	0.1022	75.64	12.86
$C_{18}H_{36}O_2$	—	—	75.98	12.76

These data agree quite well with those for stearic acid.

(b) *Isolation of unsaturated fatty acids*

The saturated fatty acids could be removed by the ether lead soap method.

The remaining ether solution of lead soap was transferred next into a separatory funnel, and acidified slightly with hydrochloric acid: fatty acids thus freed were washed with water several times, then dehydrated with anhydrous sodium sulphate. After the removal of ether, 45.8 grams of dark brown, oily, unsaturated fatty acids were obtained.

The general characteristics of these unsaturated fatty acids were as follows:

Specific gravity (at 15°C.) 0.9675
Iodine value 95.84
Neutralization value 187.95

Twenty grams of unsaturated fatty acids thus obtained were dissolved in 170 cc. of ether and cooled to −5°C.; bromine water was then added, but no crystal was formed even after 20 hours. There was therefore evidently no octabromide. The solution was then poured into a separatory funnel, excessive bromine was removed by washing with dilute sodium thiosulphite solution. Sodium thiosulphite was then removed by washing with water. Then, the preparate having been dehydrated with anhydrous sodium sulphate, ether was finally driven

off. It was next treated with petroleum ether (B.P. 40–50°C.). Although nearly all of it was dissolved, a dark brown syrnpy substance remained undissolved; the ether layer was then decanted off, and the undissolved material was washed several times and dried in a vacuum desiccator. The yield was 0.39 gram. The bromine content was determined by the Baubigny u. Chavanne method[19]. The results are as follows:

Sample g.	AgBr g.	Br %
0.2243	0.2805	53.23
0.1675	0.2102	53.43
$C_{18}H_{32}O_2Br_4$	—	53.33

These results agree perfectly with the contents of tetrabromstearic acid; linoleic acid must therefore exist in the sample.

From the petroleum ether soluble fraction, petroleum ether was evaporated almost to dryness and kept for 3 weeks at −7°C. Small quantities of white crystal resulted. On filtration of the solution and purification of the crystal, 0.62 gram was obtained.

This substance has a melting point of 112°–114°C., and on determination of its bromine content, the following results were obtained.

Sample g.	AgBr g.	Br %
0.2553	0.3185	53.55
$C_{18}H_{32}O_2Br_4$	—	53.33

The crystal agrees with tetrabromstearic acid.

After the separation of tetrabromide from the filtrate petroleum ether was completely evaporated and 29.4 grams of a brown, transparent oily substance were gained.

On determining the bromine content, the following resulted:

Sample g.	AgBr g.	Br %
0.2635	0.225	36.32
$C_{18}H_{34}O_2Br_2$	—	36.18

The substance agrees with dibromstearic acid. The existence of oleic acid is consequently obvious. Twenty nine and four-tenths grams of dibromstearic acid corresponds, when calculated as oleic acid, to 19.48 grams; the content of oleic acid in the unsaturated fatty acid therefore amounts to 94.98%.

(4) Isolation of phosphatids

In 98 grams of the ether soluble matters there were 1.5 grams of

acetone insoluble matter. This was again dissolved in a little ether and precipitated with acetone, then filtered and treated with absolute alcohol. Thus two fractions were separated, the one soluble in alcohol, and the other insoluble.

The fraction soluble in absolute alcohol was then precipitated by the addition of a large quantity of acetone, and the precipitate dissolved in ether. Then, repeating the procedure several times, precipitation by acetone and dissolution by alcohol, 0.78 gram of a white waxy powder was finally obtained.

This substance contains phosphorus and nitrogen, and its water solution is colloidal. In alcoholic solution it will be precipitated by a *chloride solution of cadomium or platinum. The nitrogen and the phosphorus* were determined by the micro-Kjeldahl method and by Neumann's method respectively. The results are:

Phosphorus 1.90% Nitrogen 1.81% P : N = 1.0 : 2.03

From these data it is apparent that this substance must be a diamino-monophosphatid.

The fraction insoluble in absolute alcohol was again dissolved in a small quantity of ether and a large quantity of alcohol was added. Forty one-one hundredths gram of a white powder was obtained.

The substance thus obtained has the general characteristics of phosphatid. The content of phosphorus and nitrogen was:

Phosphorus 3.25% Nitrogen 1.51% P : N = 1.0 : 1.04

This substance is therefore a monoamino-monophosphatid.

(5) Summary

By chemical analysis of the *Rhizopus japonicus* cultivated in Raulin's solution, 1.08 grams of ergosterol, 2.55 grams of fungisterol, 7.6 grams of palmitic acid, 1.16 grams of stearic acid and 1.19 grams of phosphatids were found per 1 kg. of dried fungous bodies. The unsaturated fatty acids are mainly oleic acid; the existence of a small quantity of linoleic acid was proved.

CHAPTER III

ALCOHOL SOLUBLE MATTERS

The content of the alcohol soluble matters in the fungous bodies is 4.635%.

(A) ALCOHOL AND SACCHARIDES

(1) *Isolation of mannit*

Nine hundred grams of the fat free fungous body were extracted for 5 days with 2.5 liters 95% alcohol and filtered. (1/5 volume of this filtrate was used for the detection of vitamin C). The residue was then extracted three times with two liters 85% alcohol at 85–90°C. for 10 hours. Both of these filtrates and wash alcohol were mixed and cooled. Abundant crystallized precipitates appeared. Recrystallized in methyl alcohol several times, and washed with alcohol and ether, 16.3 grams of white acicular crystals were obtained.

This crystal is very soluble in water, but with difficulty soluble in alcohol and quite insoluble in ether; its taste is sweet, and the melting point lies at 166°C. By elementary analysis and the determination of the refractive index, the following results were obtained.

Elementary analysis

Sample g.	CO_2 g.	H_2O g.	C %	H %
0.1254	0.0356	0.4482	7.74	39.80
0.1422	0.0400	0.5120	7.67	40.00
$C_6H_{14}O_6$	—	—	7.72	39.54

Refractive index

Five per cent boric acid solution; tube length, 10 cm.; temperature, 25°C.; Ventzke sugar scale, 3.95; corrective coefficient, 0.3465.

$$[\alpha]_D^{25°} = \frac{3.95 \times 0.3465 \times 100}{1 \times 5} = 27.3$$

From these results it is clear that this acicular crystal is mannit.

After the separation of the mannit, alcohol was removed from this alcohol extract and it was dissolved in water. After the removal of all ether soluble matters in this solution by shaking it several times with ether, it was concentrated at 40°C. Then neutral and basic lead acetate solutions were added; the precipitates thus resulting were filtered off. The lead in the filtrate was removed by hydrogen sulphide and the acetic acid by ether and calcium carbonate. The solution was then kept in a cool place for several days. Sulphuric acid was added to the concentration of 5% and saturated phosphotungstic acid solution was poured in. The precipitates thus resulting were separated. The filtrate was treated as usual, and several times decolorized with animal charcoal,

condensing to a syrupy state. One hundred and thirty five grams of syrup were obtained.

(2) *Characteristics of the syrup*

1. Water: 53.25%. 2. Solid matters: 46.75%. 3. Reducing sugar (as glucose): 6.72% (14.38% for solid matter). 4. Reducing sugar, after the syrup was hydrolized (as glucose): 28.25% (61.08% for solid matter. 5. Molisch's reaction for the carbohydrates: positive. 6. Seliwanoff's reaction and Pinoff's reaction for ketose: positive. 7. Iodine reaction for starch and dextrine: negative. 8. Aniline acetate reaction for furfurol: negative. 9. Mucic acid was not formed by keeping 1 g. of syrup dissolved in 20 cc. of nitric acid of sp. gr. 1.15 and concentrated on a water-bath with occasional stirring for several days in a cool place. 10. The above solution, after the removal of nitric acid by heating on the water-bath until it was almost dried up, was cooled. Potassium carbonate was added to saturate, then acidified by adding acetic acid drop by drop, and kept in a cool place. Abundant white crystals were obtained. On examination through the microscope the crystal seems to be potassium saccarate. 11. By excessive addition of acidic solution of phenylhydrazin to the 5% aqueous solution of the syrup a precipitate was made after a day in a cool place. The precipitate was washed with a mixed solution of ether and alcohol, recrystallized from 80% alcohol. The crystal is identical with that of mannose phenylhydrazone, its melting point being 196°C. After the removal of mannose phenylhydrazone, the filtrate was heated for 30 minutes at 100°C. on the water-bath. Abundant yellowish acicular crystals resulted. After recrystalization in alcohol, the melting point was estimated. It was 204°C., which quite agrees with that of glucosazone.

(3) *Isolation of trehalose*

Munz[6] proved the presence of trehalose in *Mucor*, and, as stated, the reducing power of the syrup after hydrolysis is very different from that before hydrolysis. One may therefore suppose the presence of trehalose in the syrup. Its isolation was consequently attempted.

One hundred grams of the syrup were dissolved in a small quantity of 95% alcohol, and kept cool (at about 10°C.) for 3 weeks. Some crystals appeared. Recrystallized several times from alcohol, 0.26 gram of white crystals was obtained.

This white, rhombic crystal melts at 203°C., reducing Fehling's

solution as sucrose. However, when the crystals are heated in 5% sulphuric acid solution for 6 hours, they do not reduce Fehling's solution; the refractive index is;

One per cent aqueous solution, tube length, 10 cm.; temperature, 20°C.; Ventzke sugar scale, 5.7; corrective coefficient, 0.3465.

$$[\alpha]_D^{20^\circ}=\frac{5.7\times0.3465\times100}{1\times1}=197$$

From these results, the crystal is probably trehalose.

(4) *Detection and isolation of saccharose*

After the removal of trehalose, alcohol was evaporated from the solution under diminished pressure at low temperature. As its reducing power is changed by hydrolysis, there must exist an appreciable quantity of unreduced sugar; an attempt was therefore made to isolate saccharose by Schultze's method[20]. Mixing with saturated strontium hydroxide solution, strontium bisaccharate was obtained. Suspended in water, strontium was removed by CO_2 gas; then when sufficiently concentrated, tiny crystal particles of saccharose were added. The crystal particles soon grew larger. The solution was filtered after several days, washed several times with absolute alcohol, and dried. Eight and six-tenths grams of white crystal were thus obtained.

This white, very sweet crystal melts at 183°C. It does not reduce Fehling's solution directly, but it reduces the reagent after hydrolysis. Seliwanoff's reaction is positive. The refractive index is as follows:

Two per cent aqueous solution; tube length, 10 cm.; temperature, 20°C.; Ventzke sugar scale, 3.8; corrective coefficient, 0.3465.

$$[\alpha]_D^{20^\circ}=\frac{3.8\times0.3465\times100}{1\times2}=65.8$$

Therefore the crystal is quite certainly saccharose. To verify this, its hydrolysis products were examined. Five grams of the crystal were dissolved in 50 cc. of water, 5 cc. of 0.279% hydrochloric acid solution were added, and the solution heated for 30 minutes on a water-bath. It was then neutralized and made up to 100 cc. with water. This hydrolyzed solution forms a brownish, black ring at the contact surface when poured gently on to concentrated sulphuric acid. Seliwanoff's reaction was also notably positive, the refractive index being—

Five per cent aqueous solution; tube length, 10 cm.; temperature, 19°C.; Ventzke sugar scale, −2.9; corrective coefficient, 0.3465.

$$[\alpha]_D^{19^\circ}=\frac{-2.9\times0.3465\times100}{1\times5}=-20$$

Two grams of phenylhydrazine hydrochloride and 3 grams of sodium acetate were added to 20 cc. of the above hydrolyzed solution and heated for 30 minutes on a water-bath. The abundant acicular crystalline precipitates thus obtained were recrystallized from alcohol. They melt at 205°C., and when put under the microscope, the characteristic acicular crystals of glucosazone were observed.

From these results it is clear that the substance is certainly saccharose, since it consists of ketose and aldose as shown. The refractive index on hydrolization shows invertose. Furthermore, glucosazone was obtained.

(5) *Detection of fructose, glucose and mannose*

After the separation of trebalose from the filtrate, excess of strontium was removed and condensed to about 70 grams. The syrup thus obtained yields both Seliwanoff's and Pinoff's reactions. The presence of fructose being obvious, the methylphenylosazone of fructose was found by Neuberg's method[21]. Ten grams of the syrup were dissolved in small quantities of water, 6 grams of methylphenylhydrazine and alcohol were added, and, when the mixture was cooled after heating, reddish orange coloured crystals were obtained. Recrystallized several times in 10% alcohol containing several drops of pyridine, 0.35 gram of the crystal, which melts at 156–158°C., was obtained. Since Neuberg showed that the melting point of the methylphenylosazone of *d*-fructose when crystallized in 10% alcohol, is 158°C., it is to be inferred that the crystal is probably methylphenylosazone of *d*-fructose.

If it is assumed that the fructose was completely osazonized, the fungous bodies contain 0.154% of fructose.

From the qualitative tests above mentioned, glucose and mannose seem to exist in the syrup. But other sugars may also exist. The diphenylhydrazone of glucose was therefore made according to Fischer's[22] and Stahel's method[23].

Twenty grams of the syrup were dissolved in a small quantity of water and 20 cc. of alcoholic solution of diphenylhydrazine were added. As the solution showed some turbidity, it was filtered and kept at room temperature for several days. A colourless prismatic crystal appeared. Recrystallized from warm water several times, washed with ether, and

dried, 2.8 grams were obtained. It melts at 161°–162°C. corresponding perfectly with diphenylhydrazone of glucose.

Moreover, by excessive addition of acidic solution of phenylhydrazine to 20 cc. of 5% aqueous solution of the syrup a precipitate was made after a day in a cool place. The precipitate was washed with a mixed solution of ether and alcohol, recrystallized from 80% alcohol. Thus 0.223 gram was obtained. The crystal is identical with that of mannose phenylhydrazone, its melting point being 196°C. Hence the content of mannose in the fungous bodies must be 1.403%.

(6) *Summary*

Rhizopus japonicus Vuillemin cultivated pure in Raulin's solution contains 16.3 grams of mannit, 0.26 gram of trehalose and 8.6 grams of saccharose in 1 kilogram of dried bodies; in addition to this, it contains 0.154% of fructose, 1.403% of mannose, and about as much glucose. A great deal of sucrose separated from the sample seems to have been transported from the culture solution.

(B) SEPARATION OF ORGANIC BASES

The precipitate formed by phosphotungstic acid was made sludgy with a little water, mixed with excess of barium hydroxide, and distilled under negative pressure; the distillate was introduced into a dilute hydrochloric acid solution, the volatile base being thus absorbed in it. The solution was then concentrated and afterwards dried in the vacuum desiccator. Some crystalline substance was recognized which does not dissolve in absolute alcohol. It was therefore recrystallized with water, and 0.15 gram of a white crystal was obtained.

The nitrogen content of this crystal was determined. It resembles that of ammonium chloride.

Sample g.	Nitrogen g.	Nitrogen %
0.012	0.0031	26.08
0.015	0.0039	26.07
NH_4Cl	—	26.05

(1) *Adenine*

The residue after the removal of the volatile base, was treated as usual; the free base solution was concentrated, acidified with nitric acid, and mixed with silver nitrate solution. A small quantity of a brownish precipitate was formed. This precipitate was washed several times with dilute nitric acid solution, and suspended in water; a slow

current of H_2S gas was passed, thus removing the silver. Then, with phosphotungstic acid, precipitation was caused as usual. From this precipitate, a free base solution was prepared after the usual treatment, and conceutrated under negative pressure till almost dry; treated with 10% ammonia solution, the greater part was dissolved. The solution was filtered and the ammonia removed by acidifying with dilute hydrochloric acid and mixed with a saturated solution of picric acid, by which a small amount of picrate was obtained.

This picrate is a beautiful yellowish acicular crystal, quite characteristic of adenine picrate. Its yield was 0.13 gram, its melting point 285°C. The picric acid content was determined, the result of which coincides with that for adenine picrate, as the next table shows.

Sample g.	Picric acid g.	Picric acid %
0.045	0.0280	62.22
0.050	0.0312	62.40
$C_5H_5N_5 \cdot C_6H_3N_3O_7$	—	62.34

(2) *Hypoxanthine*

The filtrate, after the separation of adenine picrate, was shaken several times with ether, to remove the picric acid contained therein. It was then concentrated, dissolved in a small quantity of water, and made alkaline with ammonia. Precipitation was caused by the addition of ammonia silver nitrate solution. The precipitate was collected on filter paper, washed several times with ammonia water, and boiled with nitric acid of specific gravity 1.1, filtered and cooled. The filtrate was mixed with some hot water. By passing an H_2S current through, the silver in this solution was removed. The filtrate was again slightly alkalized by the addition of ammonia, concentrated, and kept at room temperature. A white precipitate was formed.

The substance thus obtained is a white amorphous powder which has no definite melting point, but carbonizes easily. The yield was 0.076 gram; the nitrogen content in it agrees quite well with that of *hypoxanthine*.

Sample g.	Nitrogen g.	Nitrogen %
0.035	0.0144	41.17
0.040	0.0165	41.14
$C_5H_4N_4O$	—	41.25

(3) *Histidine*

Of the above mentioned filtrate from ammonia silver nitrate, after

the removal of the precipitate caused by silver nitrate, one part was used for the detection of vitamin B. To the other part an excess of silver nitrate solution was added. It was saturated with barium hydroxide, and a considerable quantity of dark brownish precipitate was formed. The precipitate was washed with barium hydroxide solution, and decomposed by nitric acid and hydrochloric acid. A brown coloured solution was thus obtained; a free basic solution was then made in the usual way. With mercurous chloride solution some precipitate was obtained. Washed with mercurous chloride solution, and suspended in water, mercury was removed by H_2S. By treating as usual, a free base solution was obtained; then, neutralized with hydrochloric acid, the solution was concentrated, and kept in a vacuum desiccator. A white crystal was obtained. It showed the diazo-reaction after Pauli's method. The strongly yellowish, acicular crystal melts at 86°C. It is therefore apparently histidine picrate. The yield was 0.027 gram and the estimation of picric acid content gives the following result.

Sample g.	Picric acid g.	Picric acid %
0.022	0.0131	59.55
$C_6H_9N_3O_2 \cdot C_6H_3N_3O_7$	—	59.64

The filtrate obtained by filtering the precipitate formed by the addition of mercurous chloride was treated with H_2S gas to remove the mercury. The free base solution was then made by the usual method, neutralized with nitric acid, concentrated, and kept in a vacuum desiccator. No crystallization was observed, however. The substance was then dissolved in water and sodium picrate added, but no arginine picrate was obtained.

The filtrate obtained by filtering the brown precipitate resulting from the addition of silver nitrate and barium hydroxide, and used for the detection of histidine, was treated with hydrochloric and sulphuric acid to remove the excess of silver and baryta. The free base solution was concentrated at a low temperature and acidified with hydrochloric acid, kept in a vacuum desiccator; then a considerable amount of prismatic crystaline mass was obtained with some adhesive matter. By treating these substances with cold absolute alcohol, soluble and insoluble parts were separated. From the insoluble part betain was obtained and from the soluble part stachydrin, as shown below.

(4) *Betain*

The chloride which does not dissolve in cold absolute alcohol was extracted with pure methyl alcohol. After evaporation some crystals were found. Recrystallization was carried out several times with methyl alcohol. Fourteen-one hundredths gram of colourless tabular crystals was obtained.

The crystal melts at 228°C., and forms with picric acid a yellowish prismatic crystal of picrate. This is decided to be betain picrate since it melts at 183°C. The content of picric acid was as follows.

Sample g.	Picric acid g.	Picric acid %
0.180	0.1190	66.11
0.220	0.1455	66.16
$C_5H_{11}NO_2 . C_6H_3N_3O_7$	—	66.18

(5) *Stachydrin*

To the fraction soluble in cold absolute alcohol, a saturated alcoholic solution of mercurous chloride was added. Precipitation occurred. After it was kept overnight at room temperature the precipitate was separated. It was filtered, concentrated after being acidified with hydrochloric acid, and kept in a vacuum desiccator. No crystal resulted, although a small quantity of adhesive material was recognized. These materials were therefore dissolved in alcohol from which the double salt of gold chloride was prepared; 0.012 gram of prismatic crystal was obtained.

This salt melts at 224°C. and by the estimation of its gold content it is recognized as the double salt of stachydrin gold chloride.

Sample g.	Gold g.	Gold %
0.011	0.0045	40.82
$C_7H_{14}NO_2 . AuCl_4$	—	40.79

(6) *Summary*

By the chemical analysis of *Rhizopus japonicus Vuillemin* cultivated with Raulin solution, the following compounds were isolated from 1 kg. dried fungous bodies: ammonium chloride, 0.15 gram; adenine picrate, 0.13 gram; hypoxanthine 0.076 gram; histidine picrate, 0.027 gram; betain picrate, 0.14 gram; stachydrin gold chloride, 0.012 gram.

(C) VITAMINS

(1) *Vitamin A*

To determine the presence of vitamin A, the following experiments

were made:

Methods and samples employed

As the subject for the experiments, healthy albino rats each weighing 30–60 grams, were employed. All these rats were separately bred in a cage of wire netting. The cages were always kept clean and much attention was paid to ventilation and sunlight. As the basal diet, Euler's vitamin A free diet[24] modified a little by the author, was used. The composition is as follows:

Casein	18 grams
Lard	10 "
Starch	60 "
McCollum's salts (185)	5 "
Oryzanin	5 "
Lemon juice	2 "
Irradiated ergosterol	0.002 mg per day.

Purification of casein

To the casein of pharmacopoeia, three times the amount of 95% alcohol were added, and boiled in a flask equipped with a reflux condenser at 88–95°C. for 10 hours every day for 5 successive days. After filtration, the casein was shaken with ether, dried in a thermostat of 80°C., and pulverized.

Purification of starch

Starch bought in the open market was repeatedly washed with water and mixed with three times its volume of 95% alcohol, shaken for 10 hours, left overnight, and decanted. This procedure was repeated five times. The starch was then extracted with ether several times and dried in a thermostat at 80°C.

Purification of lard

To the market lard, three times the volume of 95% alcohol was added, and heated in a flask equipped with a reflex condenser at 80–85°C. for 10 hours. When cooled, the upper liquid and floating sediments were rejected. These procedures were repeated several times until the solution became colourless. Keeping it in a thermostat of 80°C., the alcohol was completely evaporated.

Vitamins

As vitamin B, oryzanin was used; as vitamin D, ergosterol isolated from *Aspergillus oryzae;* and as vitamin C, lemon juice.

Casein, starch, lard and salts were mixed together in the proportion shown above. The same volume of water was added to it, and boiled on a water-bath. Then, mixed with vitamins B, C and D the preparation was given to the animals in dumpling form. The weight of the animals was examined at the same hour every day. The feeding samples to be examined for their vitamin contents were given *per os.*

Sample

The residual solution obtained by the removal of phosphatids and sterols from the ether soluble matters was used for the test of vitamin A.

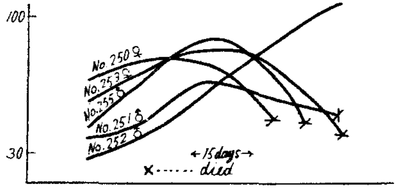

Fig. 1.
The results of feeding *Rhizopus japonicus* extract to rats

The results are shown in Fig. 1. While Albino rat No. 252, which was fed on the basal diet and carrots grew very healthily even after 2 months, two rats No. 250 and No. 255, which were fed on the basal diet and water, gained weight only at the beginning. After 3 weeks they ceased growing. Finally they began to lose weight every day, and both died before 65 days had elapsed. No. 253 was fed on the basal diet, water, and 0.1 cc. of the sample daily, which corresponds to 5 grams of the original dried fungous bodies. No. 251 was fed quite similarly, only the given quantity of the sample being different, 0.2 cc. every day, that is, corresponding to 10 grams of the original dried fungous bodies. These two rats showed quite the same results as rats Nos. 250 and 255, which were fed on the basal diet only. They died after 66 days.

From these results, it is to be concluded that *Rhizopus japonicus* cannot synthesize vitamin A.

(2) *Vitamin B*

A part of the residual solution from which adenine had been separated, was employed as the material in examining for the presence of vitamin B.

Nielsen(9) has reported recently that *Rhizopus suinus* synthesizes a substance "Rhizopin," which promotes the growth and the fermentative activities of yeast. Therefore in *Rhizopus japonicus* also the same substance may be synthesized. To examine this point, the following experiments were carried out.

Effect of the sample upon the multiplication of yeasts.

Two species of yeasts, *Saccharomyces sake* and *Saccharomyces cerevisiae* were inoculated in the smallest possible quantities in the Raulin solution. In the same medium containing 1/100 cc. of the sample per 10 cc., these were incubated in a thermostat at 33°C. for 24 hours. The number of the cells in the solutions was counted every 3 hours as graphically shown in Fig. 2. While the number of the cells in 1 cc. of Raulin's solution inoculated with *Saccharomyces cerevisiae* and *S. sake* was 4,000,000 and 18,000,000 respectively, at the end of 24 hours in the medium with the tested sample there were 76,000,000 of the former and 114,000,000 of the latter. Both of these yeast species grew far more vigorously in Raulin's solution with the tested sample.

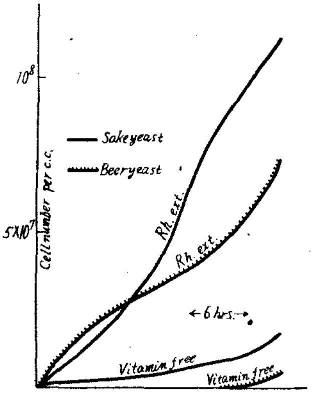

Fig. 2.
The effect of *Rhizopus japonicus* extract on the growth of yeasts

From these results, one may conclude that there is some substance in the sample which vigorously promotes the growth of the yeast.

Effect of the sample upon the growth of rats

Vitamin B is divided into three fractions from its function; the growth promoting factor for yeast, the antineuritic, and the growth accelerant. These three, however, exist, in most cases combined; as the factor which promotes the growth of the yeast has been proved, the other two factors must certainly be contained in the sample. Therefore, for the presence of growth promoting factor in the tested sample healthy white rats weighing 30–50 grams were experimented with. For the antineuritic factor, five cocks of 1300–1500 grams were employed.

The basal diet employed for the rats was of the following composition, each of the complements having been purified as already stated;

Caseiu	18 grams
Lard	10 "
Cod-liver oil	2 "
Starch	65 "
McCollum's salts (185)	5 "
Lemon juice	2 cc. per day

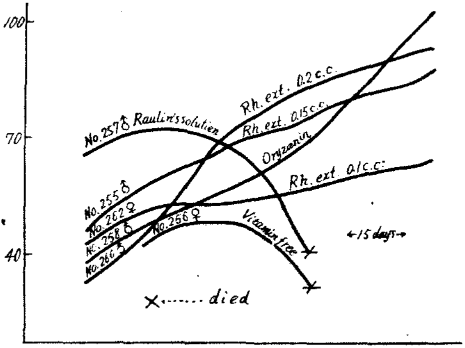

Fig. 3.
The effect of *Rhizopus japonicus* extract on the growth of young rats

The results are shown in Fig. 3. Animal No. 256, which was fed on the basal diet alone, did not grow at all and died after 43 days. No. 257, which was fed on the basal diet and 10 cc. of Raulin's solution, did not

grow well and died after 57 days, just as in the former case. But No's. 262, 255 and 260, which were raised with the basal diet and 0.1, 0.15 and 0.2 cc. of the sample for 3 months, grew excellently, even up to the end of 3 months. Compared with rat No. 258, which was fed on the basal diet plus 0.1 gram oryzanin, no great inferiority was observed.

Efficiency in curing neuritis in domestic fowls

The basal diet in the above experiment contains no vitamin B. Still, those rats which took the solution prepared from fungous bodies were able to grow well without suffering from any neuritic disease. This fact shows that the *solution contains two factors of vitamin B, the* growth promoting and the antineuritic. To ascertain the presence of the antineuritic factor and to determine its content, the following experiment was made with some cocks.

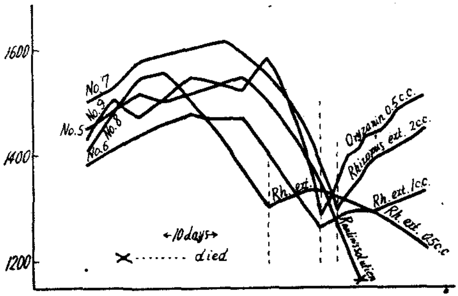

Fig. 4.
The curative effect of *Rhizopus japonicus* extract upon polyneutritis of Cocks.

Five cocks, No's. 5–9, weighing 1300–1500 grams, were fed with polished rice and water. To one of these five, No. 5, 10 cc. of Raulin's solution was compulsorily given daily. In seven weeks all these five showed typical neuritic symptoms. Now to cock No. 5, the Raulin soltion given was doubled, but without any effect whatever. It died after 8 days. But Nos. 6 and 7, to which 1 or 2 cc. of the sample were given daily, increased their weight even from the next day, and began to walk almost normally. Day after day their weight increased rapidly,

and they walked about quite freely. No. 8, however, which took only 0.5 cc. of the sample was not able to recover its health completely and after showing a slight increase of weight for one week, it began to decrease in weight day by day. Compared with No. 9, which took daily 0.5 cc. of oryzanin, No. 7 to which 2 cc. of the sample was given, *showed the same effect. Clearly, in the tested solution there exists an* antineuritic vitamin about one fourth of that of oryzanin.

(3) *Vitamin C*

Guinea pigs were fed daily on the following basal diet: 10–15 grams of oats, and the same quantity of wheat bran and 40–50 grams milk autoclaved for one hour at 120°C.

Guinea pigs, thus nourished will suffer from scurvy in two weeks and generally die in three weeks, as already reported[25]. The substance to be tested for the existence of vitamin C was therefore added to the basal diet from the beginning of the experiment. As this material 95% alcohol extract of the fungous bodies was used.

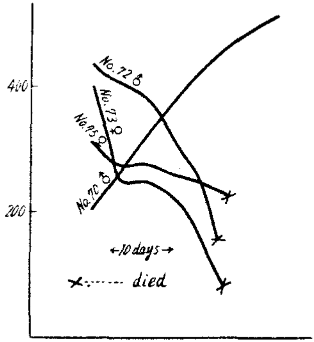

Fig. 5.
The results of feeding *Rhizopus japonicus* extract to guinea pigs.

Of four normal healthy guinea pigs, No. 72, which was fed on the basal diet only, and No's. 73 and 75 to the diet of which 2 cc. or 5 cc. of the sample were given daily, decreased in body weight every day and died in 3 weeks just as did No. 72, which had been raised on the basal diet alone, all showing the symptoms of scurvy.

From this experiment it is proved that vitamin C is not present *in the fungous body.*

(4) *Discussion*

From the above experiments, it is evident that vitamins A and C are not present in the fungous bodies, but that vitamin B is. It stimulates the growth of yeast, accelerates the growth of the animal,

and acts as an antineuritic agent.

Now the fungous bodies were obtained from the *Rhizopus japonicus*, in the vitamin-free Raulin's solution. Attention must be called to the fact that 20 generations had been successively cultured in the vitamin-free Raulin's solution. *Rhizopus japonicus* must therefore have the power of synthesizing vitamin B in Raulin's solution. It cannot, however, synthesize either vitamin A or C.

(5) *Summary*

Rhizopus japonicus Vuillemin synthesizes in Raulin's solution neither vitamin A nor C. Vitamin B, however, can be synthesized very well.

(D) *Conclusion*

One kg. dried fungous bodies of *Rhizopus japonicus Vuillemin* cultivated with Raulin's solution was extracted after the removal of all ether soluble matter with 95% alcohol. From this alcohol extract the following substances were isolated:

Mannit	16.3 grams.
Sucrose	8.6 "
Trehalose	0.26 "
Mannose	14.03%
Fructose	1.54 "
Glucose	an appreciable amount.
Ammonium chloride	0.15 grams.
Adenine (picrate)	0.13 "
Hypoxanthine	0.076 "
Histidine (picrate)	0.027 "
Betain (hydrochloride)	0.14 "
Stachydrin (gold chloride)	0.012 "

Vitamin A in the residual solution was not obtained after separating phosphatids and sterols from ether extract. Vitamin B was found in sufficient quantity in the residual solution after filtering off the adenine. Vitamin C was not found in the extract by 95% alcohol of fungous bodies formerly treated with ether.

CHAPTER IV
PROTEINS

(1) Determination of soluble proteins in water, sodium chloride, alcohol and alkali solution

To examine the distribution of nitrogen, ten grams of the dry fungous body were extracted successively with 1) water, 2) 10% sodium chloride solution, 3) 70% alcohol, 4) 0.2% NaOH solution. The nitrogen in each fraction was determined by the Kjeldahl method.

TABLE VII
The distribution of nitrogen

Fraction	% in the sample	% in dry matter	% of total nitrogen
Water soluble nitrogen	1.447	1.560	27.984
10% NaCl soluble nitrogen	0.544	0.575	10.307
70% alcohol soluble nitrogen	0.058	0.061	1.098
0.2% NaOH soluble nitrogen	0.915	0.967	17.336
Residue	2.284	2.413	43.274

As shown by the above table, the amount of nitrogenous compounds soluble in 70% alcohol is extremely small compared with the others. The amount is increased in the order of 10% NaCl solution soluble, 0.2% NaOH solution soluble and the water soluble.

(a) *Nitrogenous compound soluble in water*

The amount of nitrogen soluble in water is the largest. To study its chemical nature, it was examined with the water extract, with the following results.

1. Acetic acid does not produce a precipitate.
2. Neutralized with NaOH solution and heated there is no coagulation whatever.
3. One percent $CuSO_4$ solution does not produce a precipitate.
4. Trichloracetic acid does not produce a precipitate.
5. Tungstic acid produces a precipitate, the nitrogen content of which is only 0.552%.
6. Nessler's reagent gives a brownish precipitate.

These results show that there is no albumin in the water extract, and the nitrogen in it is all inorganic. It must have come to the fungous bodies from the culture solution.

(b) *Ten percent NaCl solution soluble protein*

Of the total nitrogen 10.307% is contained in the fraction extracted

with 10% NaCl solution; this may be globulin. But, as shown in the next table, dilution with distilled water causes no precipitation at all, a fact which shows that either there is no globulin whatever or that it is in very small quantity.

TABLE VIII
The degree of turbidity of sodium chloride solution of protein for different quantities of water

10% NaCl solution extract (cc.)	Water (cc.)	Turbidity
1	1	—
1	2	—
1	4	—
1	6	—
1	8	—
1	10	—
1	12	—
1	16	—
1	20	—
1	24	—
1	28	—
1	30	—

Now, to ascertain whether a small quantity of glubulin existed, a similar extract of 1 kilogram of the sample was dialysed in a bladder membrane for two weeks. A precipitate appeared, which showed all the colour reactions of protein and contained 15.68% nitrogen of dried matter. This substance may be considered as a globulin. Hence a small quantity of globulin seems to exist in the sample.

(c) *Seventy percent alcohol soluble protein*

As described above, the nitrogen contained in the fraction extracted with 70% alcohol was very small in quantity and comprised only 1.098% of the total nitrogen. Further studies were consequently not made.

(d) *NaOH solution soluble protein*

The nitrogen content in the extract with 0.2% NaOH solution is very high. To determine the best concentration of NaOH for extracting nitrogenous compounds from the fungous bodies and to get the largest amount of precipitate from it with acetic acid, the following experiments were tried. In four bottles containing 20 cc. of 0.1, 0.15, 0.2 and 0.25% NaOH solution respectively 2 grams of the fungous bodies were shaken for an hour with a shaking machine. The nitrogen content in each

extract was determined, and then the remainder was acidified with acetic acid. The resulting precipitates were filtered, dried and weighed. The nitrogen contents of these filtrates were also determined.

The results are shown in the following tables.

TABLE IX

Solubility of the sample in NaOH solution of different concentrations

	% in air dried matter	% in anhydrous matter	% in total nitrogen
0.1% NaOH soluble nitrogen	2.661	2.809	50.417
0.15% " " "	2.697	2.849	51.099
0.2% " " "	2.742	2.897	51.951
0.25% " " "	2.585	2.731	50.872

TABLE X.

Nitrogen precipitated by acetic acid of the sample in NaOH solution of different concentrations.

	% of nitrogen precipitated by acetic acid	% of total nitrogen	Precipitate g.
0.1% NaOH solution	1.206	22.849	0.080
0.15% " "	1.613	30.561	0.082
0.2% " "	1.983	37.609	0.110
0.25% " "	1.899	35.999	0.933

From the above results, it can be seen that 0.2% NaOH solution extracts the nitrogenous compound best and gives larger quantities of precipitate by acetic acid. The nitrogen content in this precipitate, moreover, is highest.

(2) SEPARATION AND PURIFICATION OF THE PROTEIN

To 750 grams of the residue obtained after extracting all ether and alcohol soluble matters, 2250 cc. of distilled water were added and shaken vigorously. The mixture was left overnight at room temperature. The upper liquid part was decanted off and the residue filtered. With this residue the same treatment was repeated twice.

To the residue thus obtained 2250 cc. 10% NaCl solution were added and shaken well. The mixture was left overnight at room temperature. The upper liquid was decanted, and the same treatment was repeated. The residue was then thoroughly washed with water until it became quite free from chlorine. Two tenths percent NaOH solution was added until the whole was made up to 3000 cc. The mixture was shaken well and left overnight at room temperature. It was decanted and filtered. With this residue the same treatment was repeated three

times. All these NaOH solutions were collected and filtered several times to make quite clear. To this clear filtrate dilute acetic acid was added. The protein was precipitated as a brownish amorphous mass. It was washed with water, alcohol and ether, dried in a sulphuric acid desiccator at diminished pressure, and when dried, well crushed to a fine powder. Twenty nine and seven tenths grams of brownish powder were obtained.

(3) CHEMICAL PROPERTIES OF THE PROTEIN

The brownish powder thus prepared is insoluble in water, 10% NaCl solution and alcohol, while it dissolves very easily in alkaline solution, yielding a quite brown solution which may be precipitated with acids.

This substance shows Biuret, Xanthoprotein, Millon's and Molisch's reactions, but is negative for lead sulphite reaction. It is certainly, therefore, a kind of protein.

The water and ash contents of this protein matter were determined by the ordinary method, the nitrogen content by the usual micro-method, the sulphur by Denis-Benedict's, and the phosphorus by Neumann's micro method. The results obtained were as follows:

TABLE XI

Composition of the protein

	Water	Ash	Nitrogen	Phosphorus	Sulphur
% in air dry	10.714	0.187	13.592	1.148	0.192
% in water and ash free subst.	—	—	15.255	1.288	0.215

(4) DETERMINATION OF AMINO ACIDS

As the nutritive value of proteins is influenced by the kinds and the amounts of amino acids contained therein, the quantities of various amino acids contained in this protein must be determined. Van Slyke's method[31] was employed for this purpose but Okuda's method[32] was used for cystine estimation.

The sample was boiled for 12 hours on a sand-bath under a reflux condenser with 20 times the amount of 20% HCl. Hydrolysis completed, almost all HCl in the solution was driven off under diminished pressure at 40°C. or lower. The residue was neutralized and made slightly alkaline by the addition of 10% calcium hydroxide solution. The ammonia nitrogen liberated was distilled into a standard sulphuric acid under diminished pressure at below 40°C. The remaining fluid

was then filtered and separated from melanine nitrogen. The melanine nitrogen was determined by Kjeldahl's method. The filtrate was then acidified with HCl and evaporated under negative pressure at 40°C. or below. To the concentrated filtrate 18 cc. of concentrated hydrochloric acid and 15 grams of phosphotungstic acid were added and the diamino acids were precipitated. After 48 hours undisturbed standing, the precipitate was filtered by suction and treated with a mixture of ether and amylalcohol according to Van Slyke's modified method. The results obtained were as follows:

TABLE XII

The kinds of nitrogen found in protein

Total nitrogen	15.255%	100.00 %
Amide nitrogen	2.524 "	16.545 "
Humin nitrogen	0.623 "	4.084 "
Diamino nitrogen	3.965 "	25.991 "
Arginine nitrogen	2.375 "	15.568 "
Histidine nitrogen	1.114 "	7.302 "
Lysine nitrogen	0.471 "	3.088 "
Cystine nitrogen	0.005 "	0.033 "
Monoamino nitrogen	7.498 "	49.092 "
Free amino nitrogen	7.072 "	46.359 "
Non-amino nitrogen	0.426 "	2.733 "
Loss nitrogen	0.645 "	4.288 "

In the last column are shown the values calculated when the total nitrogen is taken as 100.

(5) DETERMINATION OF TYROSINE AND TRYPTOPHANE

For the determination of tyrosine and tryptophane contained in the protein, Folin and Loony's method[33] was employed. One gram of the protein was hydrolyzed with 3.5 grams of barium hydroxide and 25 cc. of distilled water under a reflux condenser for 48 hours. To the solution 30 cc. of 20% H_2SO_4 solution were added and heated on the water-bath for an hour. The precipitate was filtered after cooling and was then diluted with distilled water until this volume reached 100 cc.

By means of a pipette, 2 cc. of the filtrate were measured into a 15 cc. centrifuging tube graduated at a volume of 10 cc. Two cc. of 10 per cent mercuric sulphate solution were added, and the mixture was at once diluted with 5% sulphuric acid to the 10 cc. mark. A rubber stopper was inserted, and the solution was shaken vigorously to ensure thorough mixing. The tube was allowed to stand for two hours and was

then centrifuged.

The clear supernatant liquid containing tyrosine was poured into a clean dry test tube and set aside for tyrosine determination. The centrifuging tube containing the mercuric tryptophane sediment was then filled to the 10 cc. mark with 5% sulphuric acid, its own rubber stopper was again inserted, and the mixture shaken. After removing the stopper, the liquid in the container was drained for half a minute.

Tyrosine.—Five cc. of the liquid containing tyrosine were transferred into a 100 cc. volumetric flask. Into another similar flask, 1 cc. of the standard sulphuric acid tyrosine solution containing 1 mg. was introduced. To the latter, 1 cc. of the acidic mercuric sulphate solution and 3 cc. of 5 per cent sulphuric acid solution was added. Then, to each flask about 30 cc. of water, 20 cc. of saturated sodium carbonate solution, and 4 cc. of 5 per cent sodium cyanide solution were added, in the order described. Two cc. of phenol reagent were added and the whole was mixed and stood for 30 minutes. Colour comparison was made iu the usual manner. Setting the staudard at 20 mm., the reading of the sample was 13.8 mm.

Tryptophane.—To the unknown mercury precipitate and to a similarly precipitated and centrifuged standard which contains 1 mg. tryptophane, 6 cc. of water were added, and shaken so as to secure a uniform suspension. Without any unnecessary delay, 4 cc. of 5 per cent sodium cyanide were added to each, and again shaken. A complete solution was at once obtained. The standard and the unknown solution substance were each rinsed into a 100 cc. volumetric flask, keeping approximately equal volumes. Twenty cc. of saturated sodium carbonate solution were added and, finally, shaking the while, 2 cc. of the phenol reagent were added, and stood for 30 minutes. It was then diluted to the volume and colour comparison was carried out, setting the standard at 20 mm. The reading was found to be 72.7 mm.

The amounts of tyrosine and tryptophane contained in the sample are accordingly as follows:

Tyrosine .... 7.245% Tryptophane 1.435%

The above results show that although the amount of diamino nitrogen is smaller than that of monoamino, it occupies 25.991% of the total nitrogen in the isolated protein. It is also rich in arginine, and histidine, and contains suitable amounts of lysine and also a small quantity of cystine. Tyrosine and tryptophane are also contained in considerable amounts. These suggest that this protein has undoubtedly

a high nutritive value.

(6) Ratio of cystine sulphur to the total sulphur

As already described, the total sulphur is 0.216 per cent and the cystine sulphur is 0.005 per cent: therefore, the ratio of cystine sulphur to the total sulphur is 1:18.

(7) The formation of iodine-protein

As iodine combines with the tyrosine group of protein, each protein must show a different degree of combination with iodine, as shown by Blum and Strauss[37] and by Tadokoro and Nakamura[38]. The writer tried next the preparation of the iodine-protein with the isolated protein.

To 0.2 gram of the protein 150 cc. of 3.5% ammonium hydroxide solution were added. The mixture was then stirred well and stood overnight, 50 cc. of alcoholic 1/10 normal iodine solution were added and laid aside overnight. The clear yellowish solution was filtered by suction, the filtrate acidified with dilute sulphuric acid. A precipitate of iodine protein was formed. After a while, the upper solution was decanted off, the precipitate was suspended in water, dissolved by the addition of sodium hydroxide solution, and again precipitated with sulphuric acid. These treatments were repeated twice so as to remove the excess of iodine from the protein. The precipitate was then washed with water, using a centrifugal machine until no more ammonia was detected in the washing. It was then washed with alcohol, absolute alcohol and ether. The iodine-protein thus obtained was dried over sulphuric acid in a desiccator of diminished pressure. A fine, slightly yellowish powder was obtained.

By means of Baubigny and Chavanne's method[39], the iodine content of the powder was determined. Forty cc. conc. H_2SO_4, 0.1 g. powdered silver nitrate and potassium bichromate were taken into a 300 cc.-Erlenmeyer's flask, and shaken well. To the mixture was added the iodine-protein, and the whole was heated to 150–170°C. until no more oxygen was evolved. After cooling, 150 cc. of water and 10 cc. of alcohol were added, and by reduction with a saturated solution of sodium sulphite, silver iodide precipitates were obtained. These were gathered, washed and weighed in a Gooch's crucible in the usual way.

Sample g.	Silver iodine g.	Iodine mg.	Iodine %
0.113	0.034	18.369	16.167

The iodine value of serum-albumin is 8.6% according to Blum[37] and that of an oryzenin isolated by Tadokoro[38] from glutinous rice is 11.764%. Comparing the present results with these values protein shows a much higher valne, 16.167%, coinciding with the fact that tyrosine is contained in the protein to as great a quantity as 7.245%.

(8) Partial hydrolysis

The degree of condensation of proteins seems to be observed depending upon their partial hydrolysis.

Two grams of the sample were hydrolyzed on a water bath in a 100 cc. flask with 20 cc. of hydrochloric acid of different concentrations, for varying periods of from 5 to 25 hours. At the end of the required period, the contents in these flasks were filtered, and the filtrates made up to a definite volume. Ammonia nitrogen, free amino nitrogen and the substance which is precipitated by sodium tungstate were determined in the usual way. The results were as follows:

Table XIII

Results of partial hydrolysis

Concentration	Ammonia nitrogen: Time (hrs.) 5	10	15	20	25
5%	0.069	0.716	0.341	0.915	1.255
10%	0.075	0.717	0.759	1.120	1.388
20%	0.085	0.963	1.156	1.393	1.403

Concentration	Free amino nitrogen: Time (hrs.) 5	10	15	20	25
5%	0.028	0.059	0.065	0.075	0.102
10%	0.081	0.120	0.137	0.149	0.162
20%	0.114	0.125	0.165	0.175	0.223

Concentration	Nitrogen precipitated by sodium tungstate: Time (hrs.) 5	10	15	20	25
5%	3.159	2.984	2.591	2.550	3.928
10%	2.409	2.353	2.599	1.984	1.579
20%	1.646	1.822	3.793	1.700	1.241

If the total nitrogen is taken as 100, the results are as follows;

TABLE XIV
Results of partial hydrolysis

Concentration	Ammonia nitrogen — Time (hrs.) 5	10	15	20	25
5%	1.307	13.566	14.040	17.336	23.779
10%	1.427	13.567	14.380	21.220	26.297
20%	1.610	18.246	21.921	26.395	26.581

Concentration	Free amino nitrogen — Time (hrs.) 5	10	15	20	25
5%	0.531	1.118	1.220	1.421	1.952
10%	1.530	2.274	2.596	2.823	3.067
20%	2.160	2.372	3.126	3.316	4.225

Concentration	Nitrogen precipitated by sodium tungstate — Time (hrs.) 5	10	15	20	25
5%	59.663	56.343	49.091	48.314	74.422
10%	45.623	44.579	48.485	37.588	29.917
20%	31.185	34.519	71.864	32.209	23.511

From the above results it can be seen that the protein is condensed just as ordinary proteins.

(9) ISOELECTRIC POINT OF THE ISOLATED PROTEIN

Pauli and Matula[34], Loeb[35] and Michaelis[36] showed that all physical properties of proteins are maximum or minimum at their isoelectric point: minimum for osmotic pressure, viscosity, amount of alcohol required for their precipitation, electric conductivity and swelling, and maximum for turbidity and surface tension. The isoelectric *point of the protein may consequently be easily determined.* For the determination of the isoelectric point of the isolated protein surface tension, viscosity and turbidity were measured by the ordinary method. The results of this investigation were as follows:

TABLE XV
The degree of surface tension, turbidity and viscosity of alkali solution of protein for different quantities of HCl

pH value	N/100 HCl (cc.)	Surface tension (degree)	Turbidity	Viscosity (minutes)
—	1.0	74.0	—	0.49
—	1.5	72.5	—	—
—	2.0	70.0	—	0.56

—	2.5	67.5	—	—
—	3.0	65.5	—	1.17
—	3.5	63.0	—	—
—	4.0	64.0	+	1.46
—	4.5	66.0	++	—
—	5.0	67.0	+++	0.52
—	5.1	67.2	++++	—
—	5.2	67.5	+++++	0.50
—	5.3	67.8	++++++	—
3.02	5.4	68.0	++++++++++	0.48
2.95	5.5	68.0	++++++++	0.45
—	5.6	67.8	++++++++	0.49
—	5.8	67.4	++++++++	—
—	6.0	67.0	++++++++	0.59

The data show that the isoelectric point of the protein lies between pH 2.95–3.02.

(10) On the protein

It must now be determined to what class of protein this substance belongs.

Very carefully prepared and purified substance was still very brown. It was therefore supposed to be one of the chromoproteids. It was examined by Kylin's method[26]. Two grams of it were hydrolyzed in 20 cc. of 0.5% HCl for an hour at 60°C., and vigorously shaken with ether; but the ether remained colourless. When treated with alkali and pepsin, and shaken with ether and amylalcohol, the solvents also remained quite colourless. From these results it seems that this protein is not a chromoproteid.

Molisch's reaction is strongly positive,—which indicates the existence of carbohydrate. But when vegetable proteins are isolated, carbohydrate is almost inevitably admixed. Therefore this carbohydrate may be seen as only some impurity admixed. It may be thought to be due, however, to the carbohydrate radical existing in the protein molecule itself as glucoproteid.

In general, glucoproteid is easily decomposed and produces carbohydrates; therefore, if hydrolyzed by weak mineral acids, Fehling's solution can be reduced. Such reducing sugars are glucosamine, glucose, etc., and they exist sometimes directly combined with protein, but mostly as one component of some compounds which combine with protein molecules. Anyway, in the decomposition products of glucoproteid or related substances, glucosamine will always be found and

detected by Oswald's method[27], as follows.

Five grams of the protein were hydrolyzed in 150 cc. of 3% hydrochloric acid for five hours. The material was cooled and filtered. The filtrate was concentrated under negative pressure at low temperature, decolorized with animal charcoal, phosphotungstic acid solution was added, and filtered after a day. The filtrate was washed several times with a mixed solution of equal parts of amylalcohol and ether, and concentrated at 40°C. under negative pressure into a syrupy state. Some tiny crystals of glucosamine hydrochloride were inoculated in it and kept in a vacuum desiccator for several weeks. But no crystallization of glucosamine was observed. Hence this protein does not seem to belong to the glucoproteids.

The phosphorus content of this protein matter is too high for that of an ordinary protein. Hence the substance is perhaps a phosphoprotein or nucleoproteid. To determine this, the content of purine nitrogen was estimated by the precipitation method according to Krüger and Wulff[28] with copper sulphate and sodium bisulphite. One hundred cc. of 0.5% solution of this protein were boiled, 10 cc. of 50% sodium bisulphite solution and 10 cc. of 13% copper sulphate solution were added, and heated. Five cc. of 10% barium chloride solution were poured in, and left for two hours. The precipitate was combusted together with filter paper by Gunning's method, and titrated with N/100 oxalic acid solution. Four and five-tenths cc. of N/100 oxalic acid were consumed. As 1 cc. of the oxalic acid equals 0.00014 g. N, 0.5 g. of this protein contains 0.63 mg. purine base nitrogen, this is, 1.26 mg. purine base nitrogen is contained in 1 grain of the protein, or the content of the nucleic acid nitrogen in this protein is only 1.26%.

As many investigators,[29] [30] insist that this method leads generally to a rather higher value and some quantities of purine base nitrogen are always found even in nucleic acid free proteins, this protein probably contains almost no nucleic acid, and is therefore a kind of phosphoprotein.

Elementary composition of the studied protein

Elementary analysis shows the following results:

Sample g.	Carbon dioxide g.	Water g.	Carbon %	Hydrogen %
0.0772	0.1506	0.0611	53.203	8.769
0.0851	0.1662	0.0669	53.263	8.735
Average			53.232	8.752

Therefore the elementary composition of the protein, is:

Nitrogen	Hydrogen	Carbon	Sulphur	Phosphorus	Oxygen
%	%	%	%	%	%
15.255	8.752	53.233	0.216	1.288	21.256

(11) Discussion

As stated above, from 1 kg. of dried fungous bodies of *Rhizopus japonicus Vuillemin* cultivated in Raulin's solution, 29.7 grams of a certain kind of protein were isolated. This protein, which is nearly insoluble in water, sodium chloride solution and alcohol, dissolves easily in sodium hydroxide solution, is precipitated by acid, and was recognized as a kind of phosphoprotein.

The elementary composition of this protein is: nitrogen, 15.25%; hydrogen, 8.752%; carbon, 53.233%; sulphur, 0.211%; phosphorus, 1.288%; oxygen, 21.256%. It contains tyrosine, 7.245%; tryptophane, 1.435%; arginine, 2.375%; histidine, 1.114%; lysine, 0.471% and cystine, 0.005%. From the results of partial hydrolysis, the substance seems to be condensed to the degree of ordinary proteins. The ratio of cystine sulphur to total sulphur is 1:18; iodine value, 16.167%. The isoelectric point lies between pH 2.95–3.02.

Isolation of proteins from *Rhizopus*-species, is very rare. Thomas[40] separated phosphoprotein from *Aspergillus niger*, and Takada[41] recently obtained a phosphoglucoproteid from *Aspergillus oryzae*. Compared with these, the protein isolated by the author shows the following properties.

TABLE XVI

Comparison of characters of fungus protein by different authors

	Author (*Rh. japonicus*)	Takada (*Asp. oryzae*)	Thomas (*Asp. niger*)
Solubility in water	Insoluble	Insoluble	Insoluble
Solubility in NaCl solution	Almost insoluble	Slightly sol.	Partly sol.
Colour reaction for protein	All positive except PbS reaction	"	"
Carbohydrates	No. glucosamine	Glucosamine present	glucosamine present.
Nitrogen content	15.255%	14.78%	12.9%
Phosphorus content	1.288%	1.77%	0.7%
Sulphur content	0.216%	Trace	0.5%
Humin nitrogen	4.084%	3.01%	4.0%
Basic nitrogen	25.991%	29.11%	15.6%
Monoamino nitrogen	45.092%	54.21%	73.1%
Total Sulphur : Cystine Sulphur	1 : 18	—	—
Iodine-value	16.167	—	—
Isoelectric point	2.95–3.02	—	—

These three sets of figures resemble each other in respect of solubility, colour reactions and amino acid content, but the protein isolated by the author contains no glucosamin and is fundamentally different in regard to its elementary composition, iodine value, ratio of cystine sulphur to total sulphur, and isoelectric point. And as no report has been previously made on the isolation of this kind of protein from the *Rhizopus* species, the author has named this protein "Rhizopenin."

(12) SUMMARY

From 1 kg. of dried fungous bodies of *Rhizopus japonicus Vuillemin* cultured in Raulin's solution, 29.7 grams of protein were isolated.

This protein is nearly insoluble in water, NaCl solution and alcohol, while soluble easily in NaOH solution and is precipitated by acid. It is a kind of phosphoprotein, and it is proposed by the author to name it "Rhizopenin."

CHAPTER V

DIGESTIVE EXAMINATION

As described, *Rhizopus japonicus* in Raulin's solution can nutritively synthesize valuable proteins, lipid, carbohydrates and vitamins in a very short time. In order to determine the digestibility of these components, the following digestive examination was carried out. There is a large number of methods for the examination of digestibility, but recently Bergeim[42] discovered a comparatively simple method: when a diet containing ferric oxide is given to experimental animals, the ferric oxide, without being absorbed in the intestinal canals, is excreted quantitatively in the faeces; therefore if the ratio of ferric oxide to the tested components in both diet and faeces is determined, the digestive coefficient of the sample being tested is to be calculated by the following equation:

$$\left(\frac{\%\text{ of tested component in diet}}{\%\text{ of ferric oxide in diet}} - \frac{\%\text{ of tested component in diet}}{\%\text{ of ferric oxide in faeces}}\right) \times \frac{\%\text{ of ferric oxide in diet}}{\%\text{ of tested component in faeces}} \times 100.$$

By this method the digestive coefficient of the components contained in *Rhizopus japonicus* was determined.

White rats were fed with diets No. 1 (15 g. casein, 65 g. starch, 10 g. lard, 2 g. cod-liver oil, 3 g. oryzanin, 5 g. McCollum's salts, 0.5 g.

ferric oxide) and No. 2 (100 g. *Rhizopus japonicus,* 0.5 g. ferric oxide), respectively, for a week. The components of the diet and the faeces were the determined, the results being as follows:

TABLE XVII

The composition of diets and faeces

	Diet No. 1. %	Faeces No. 1. %	Diet No. 2. %	Faeces No. 2. %
Water	8.65	5.22	5.34	8.35
Nitrogen	2.84	3.51	5.28	5.62
Carbohydrates	57.22	53.20	27.50	22.32
Ashes (Fe_2O_3 not included)	3.18	8.58	4.53	5.22
Fe_2O_3	0.25	3.43	0.32	1.21

The digestive coefficients of the tested components in diets Nos. 1 and 2, calculated by Bergeim's formula, are:

TABLE XVIII

Digestibility of the fungus bodies

	Solid matters	Nitrogenous matters	Carbohydrates	Ash
Diet No. 1.	92.4	91.5	93.2	80.2
Diet No. 2.	74.1	72.1	78.6	74.2

Thus the digestive coefficients of the components contained in *Rhizopus japonicus* are, compared with those of the synthetic diet, rather lower, but all components are digested and absorbed to a considerable extent.

CONCLUSION

After 20 generations of culture in order to eliminate the effect caused by culture, the following substances were isolated from 1 kg. dried fungous bodies of *Rhizopus japonicus* cultivated for 5–7 days in Raulin's solution, which contains no organic substance other than sucrose and tartaric acid.

Ergosterol	1.08 g.
Fungisterol	2.55 g.
Palmitic acid	7.60 g.
Stearic acid	1.16 g.
Phosphatids	1.19 g.
Mannose	14.03%
Mannit	16.30 g.
Trehalose	0.26 g.
Fructose	1.54%

Glucose	an appreciable amount.
Ammonium chloride	*0.15* g.
Hypoxanthine	*0.076* g.
Betainhydrochloride	*0.14* g.
Adenine picrate	0.13 g.
Histidine picrate	0.027 g.
Stachydrin gold chloride	0.012 g.
Vitamin B	
0.2% NaOH solution soluble Protein	29.7 g.

From the above data, it is known that important nutritive materials are synthesized by *Rhizopus japonicus* in Raulin's solution in such a short time, and from its digestive coefficient of 72.1 in nitrogenous substances, 78.6 in carbohydrates and 74.2 in ashes, that these components are digested quite well.

The protein is synthesized by *Rhizopus japonicus*, which is soluble easily in sodium hydroxide solution and is precipitated by acid. It is *a kind of phosphoprotein with the following elementary composition*: nitrogen, 15.255%; hydrogen, 8.752%; carbon, 53.233%; sulphur, 0.216%; phosphorus, 1.288% and oxygen, 21.256%. Rich in basic amino acids, it is a valuable nutrient; it contains also appreciable amounts of tyrosine and tryptophane. The ratio of cystine sulphur to the total sulphur is 1:18, the iodine value, 16.167%. Its isoelectric point lies between pH 2.95 and 3.02.

As described above, the protein differs completely from those isolated from *Asp. niger* by Thomas, and from *Asp. oryzae* by Takada, though they resemble each other in respect to solubility, colour reaction and amino acid contents. The chief difference consists in our proteins lack of glucosamin content, its elementary composition, its iodine value, the ratio between cystine sulphur and total sulphur, and its isoelectric point. Consequently, the author proposes to name the studied protein "Rhizopenin," as no report concerning this protein has hitherto been published.

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ECOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE BLOOMING OF OAT FLOWERS

By

Giichi Misonoo

(With 1 Plate and 1 Text-figure)

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Introduction

Ecological and physiological studies on the blooming of cereals are very valuable and interesting problems for plant breeding and crop science from both practical and scientific points of view. RIMPAU (1882), KÖRNICKE (1885), FRUWIRTH (1905), NOWACKI (1911) and others have studied on the blooming of oats. According to the results of their studies, *blooming occurs in the afternoon* unlike other cereals. They, however, differ in their opinions concerning the time of the day when blooming begins to take place. This is generally considered to be due to the fact that the external conditions differ according to the time and place of observation.

According to the writer's observation there is a marked difference each day in the time of blooming even at the same place and on certain *days no blooming takes place at all even during the blooming period*. This is generally thought to be probably due to the fact that environmental conditions differ each day, but there seems to be no definite opinion as to what the most important factor among the external conditions is, and also as to what significance the factor has on the physiology of blooming.

The writer carried out experiments upon these problems during the past few years and in the present work wishes to show the exact

relation between blooming and external conditions and also the significance of the factor with respect to the physiology of blooming.

I. Observation on the Blooming of Oat Flowers

1. Material and Methods

The plants on which most of the observations were made were those grown 1927, 1928, 1929 in the experimental farm of the College of Agriculture, Hokkaido Imperial University. The variety used was Clydesdale (*Avena sativa* L.). In this variety the spikelets are arranged in a panicle, in which the number of whorls ranges from four to nine, mostly five or six, the number of spikelets in a panicle being nearly 60. The spiklet consists of two or three florets and is covered with inner and outer glumes of thin membrane. Each floret has a lemma and a palet in which are contained a pistil and three stamens. The pistil is made up of a single ovary and two styles, the ends of which look like feathers. At the base of the ovary there is a pair of lodicules inside the lemma. When the flower has developed and the time of blooming comes, the lemma and palet open, making a certain angle. The filament elongates out of the lemma and palet, the anther scatters pollen and at the same time the stigma reaches the stage ready to receive pollen. This is the blooming process of oats.

In order to facilitate close observations on the order of blooming of a panicle, it was fixed on a wire gauge frame. The seeds were sown at three different periods so as to be able to make observations on the blooming for the longer period (from the end of June to the middle of October).

When the flowers began to bloom, their number was recorded every 15 minutes. The lemma of those flowers which had bloomed were cut off in order to distinguish them from those which bloomed later. Both temperature and humidity were measured by means of a psychrometer which was hung in a shelter situated in the experimental farm, and the computation of humidity was made according to Nakagawa's[28] appended Table VIII. The other meteorological data, such as rainfall, velocity of wind, hours of sunshine, etc., used in this study were procured from the Sapporo Weather Station.

2. Order and Duration of Blooming

Akemine[1] studied in detail on the order and duration of the

blooming of rice, and later a number of investigators (*Iso*,[9] LAUDE,[18] YAMAGUCHI[41] and JONES[11]) made similar observations on the same plant. KÖRNICKE,[16] FRUWIRTH,[5] LEIGHTY,[17] PERCIVAL[35] and OBERMAYER[34] studied on wheat. FRUWIRTH[5] and KÖRNICKE[16] on barely, and TSCHERMAK,[38] OBERMAYER,[34] KÖRNICKE[16] and FRUWIRTH[5] on rye. Very few studies have been made on oats and the reports made by KÖRNICKE[16] and FRUWIRTH[4 & 5] seem to be the only ones. The writer observed the blooming of oats, the order and duration of blooming.

Order and duration of blooming of flowers on a single plant of oats

Of the order of blooming of oat plants, FRUWIRTH[4] reported that blooming commences with the panicle of the main stem and goes to the tillers. Of rice AKEMINE[1] states that the one which begins to bloom first of the whole plant is the head of the main stem, but that when the head of the stooling stem emerges from the sheath at the same time with that of the main stem no difference in the speed of the blooming is recognized. The writer chose 2 plants and observations were made on each plant, the results being as shown in Table 1.

TABLE 1. *Order and duration of blooming of flowers on a single plant of oats in 1926*

Plant no.	Stem no.	The time when the panicle emerges from the sheath: beginning	The time when the panicle emerges from the sheath: end	Time of blooming: beginning	Time of blooming: end	The days required to complete the blooming: panicle	The days required to complete the blooming: plant
I	1st	July 9 10 a.m.	July 14 5 p.m.	July 11	July 19	9 days	29 days
	2nd	„ 12 1 p.m.	„ 17 3 p.m.	„ 15	„ 23	9 „	
	3rd	„ 14 6 p.m.	„ 21 5 a.m.	„ 16	„ 25	10 „	
	4th	„ 18 7 p.m.	„ 25 4 a.m.	„ 21	„ 28	8 „	
	5th	„ 19 6 p.m.	„ 26 8 a.m.	„ 21	„ 29	9 „	
	6th	„ 28 7 p.m.	Aug. 3 3 p.m.	„ 30	Aug. 5	7 „	
	7th	„ 31 5 p.m.	„ 6 6 p.m.	Aug. 3	„ 8	6 „	
	8th	Aug. 5 4 p.m.	„ 11 3 p.m.	„ 8	„ ?	?	
II	1st	July 7 8 a.m.	July 13 9 a.m.	July 9	July 17	9 days	31 days
	2nd	„ 11 2 p.m.	„ 16 5 p.m.	„ 13	„ 22	10 „	
	3rd	„ 12 5 p.m.	„ 19 1 p.m.	„ 15	„ 24	10 „	
	4th	„ 20 9 a.m.	„ 26 9 a.m.	„ 22	„ 28	7 „	
	5th	„ 20 5 p.m.	„ 26 7 a.m.	„ 22	„ 29	9 „	
	6th	„ 28 7 a.m.	Aug. 2 5 p.m.	„ 29	Aug. 4	7 „	
	7th	Aug. 1 3 p.m.	„ 6 9 a.m.	Aug. 2	„ 7	6 „	
	8th	„ 4 10 a.m.	„ 9 12 p.m.	„ 5	„ 7	4 „	
	9th	„ 5 6 p.m.	„ 11 2 p.m.	„ 8	„ 11	4 „	
	10th	„ 6 10 a.m.	„ 11 6 p.m.	?	?	?	

As shown in Table 1, on each of the two plants the first flower to bloom is in the panicle of the main stem, the stooling stem following it, and blooming takes place according to the order of the emergence of the panicle from the sheath.

According to FRUWIRTH[5] it required from 11 to 14 days to complete the blooming of all the plants in the breeding nursery. It was noted by the writer to take from 29 to 31 days to complete the blooming of all panicles on a plant. In September 1927, it required over 40 days to complete the blooming of 30 plants and owing to low temperature many flowers remained unopen. But in the following summer (1928) when the blooming of 15 plants was observed it started on July 7 and was completed on July 19 requiring only 13 days. The days required for the whole plants to complete blooming, vary considerably according to the environmental conditions. The length of continuance of tillering is of course one of them, but the external conditions, especially temperature, have much to do with blooming. In a high temperature the blooming is completed in a short period of time while in low temperature a comparatively long period of time is required.

Order and Duration of Blooming of a Head

Regarding the order of blooming of the panicle, KÖRNICKE[16] states that blooming begins with the flower at the top of the panicle and extends downward.

The results of the writer's observations are similar to his as shown in Text Figure 1 and they may be itemized as follows:—

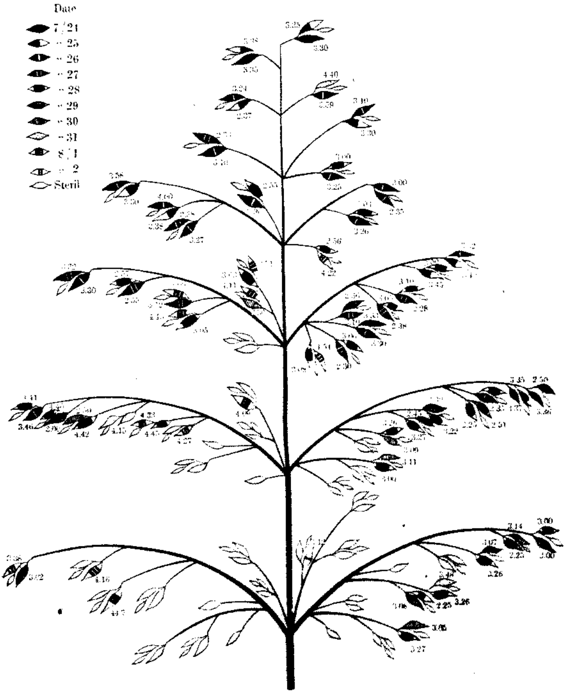
1:—The first flowers to open in the panicle are in the spikelet at the top of the main axis and the blooming goes on downwards, in the same order as reported by KÖRNICKE.

2:—When the same node has more than two branches, the flower in each branch opens according to the order of length. The flower on the longest branch blooms first and that on the shortest last.

3:—The order of blooming on a primary branch is similar to that in the panicle.

4:—The days required for completing the blooming of the entire panicle vary according to the external conditions, but when the conditions are favourable about 8 days are required.

5:—When there are more than two flowers in a spikelet, the lower flower opens first, then the second and the third in order. The intervals in the time of blooming of the first, the second and the third flower



Text-Figure 1

Order of Blooming of Oats in 1926

vary according to the outside conditions. When the conditions are favourable the first and the second bloom almost at the same time, but when they are not so, the latter blooms two or three days after.

6:—According to the writer's observations in favourable weather, the time required from opening till closing of a flower was from about 50 minutes to an hour and a half. The majority required 60–80 minutes.

On the days when the hour of blooming was generally earlier they remain open a longer time, but on the days when the flowers bloomed later they closed in a short time.

TABLE 2. *Number of flowers opened in a single panicle and the time elapsing between the beginning and the end of blooming in the day in 1926*

Date	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	av.
Number of flowers opened.	6	10	15	19	3	3	2	14	7	8.8
Blooming time of the first flower. (P.M.)	3.00	2.55	2.21	2.57	2.25	3.10	3.43	2.44	3.00	
Blooming time of the last flower. (P.M.)	3.40	3.45	3.30	3.30	2.38	3.16	4.17	5.09	4.30	
Time elapsed between the beginning and end of blooming. (minutes)	40	50	69	33	13	6	34	145	90	53.3

7:—It should be noted in the blooming of oats that when the so-called blooming time comes, all plants in the whole field begin to bloom almost at the same time. When the blooming time has not come, blooming never takes place. The beginning and the end of the blooming of the day are clearly marked. Table 2 shows the time of the first blooming on the day, and that of the last blooming and the nnmber of flowers opened on each day in a panicle.

As shown in Table 2, on the 6th day three flowers bloomed for only 6 minutes and on the 8th, 14 flowers require as long a duration as 145 minutes for blooming. That is, the average length of time in which the blooming took place was 53.3 minutes. The difference in the length of time is mainly due to the temperature changes, and on the day when the temperature rose to its highest point in the morning and then went

down, the length of time in which blooming occurred was short, but when the temperature which gradually rose went down gradually, it required a longer time.

3. TIME OF BLOOMING

According to the reports of observations made by FRUWIRTH, GODRON, HILDEBRAND, RIMPAU, NOWACKI and KÖRNICKE as to the blooming time of oat flowers they begin to open only in the afternoon, unlike rice, wheat, barley and rye which begin to open in the morning.

TANNERT alone states that the oats bloom from 9 a.m. to 3 p.m. and recognizes the blooming in the morning. But many of the other workers state that the blooming takes place in the afternoon, although their opinions regarding the time differ from one another. FRUWIRTH states that the oat flowers begin to bloom between 2 and 3 p.m. and some open as late as at about 7 or 8 p.m. in the evening. GODRON states that the blooming takes place between 2 and 4 p.m., HILDEBRAND between noon and sunset, and RIMPAU that it begins at about 4 p.m. and the most intense blooming occurs between 5 and 6 in the evening. NOWACKI states that the most intense blooming takes place between 3 and 4 p.m. and continues until about 8 p.m. in the evening. KÖRNICKE states that some open intensely between 3 and 4 p.m. and some after 8 p.m. The writer also observed the time of blooming and reported the result of the observation in his previous papers.[19 & 20] Since then for three years (1927–28–29) the writer continued his observation. Before describing the result of these observations it seems necessary to the writer to limit the meaning of the "time of blooming" as mentioned in his work. As shown in the previous chapter, the beginning and the end of the blooming of oat flowers during the day are distinctly marked and until the time of blooming arrives, not a single flower opens, but as soon as the time does come, all the flowers in the field begin to bloom almost at the same time and ordinarily the blooming goes on for about an hour or so, but after that no blooming takes place at all. Therefore the beginning and the end of blooming are quite distinct and so the writer calls the time when the first flower of the experimental plants begins to bloom the time of blooming on that day.

From the results of observations made on the time and the number of flowers opened as shown in the appended tables, the days on which blooming took place at the same time, arranged in intervals of 15 minutes are collected in Table 3.

TABLE 3. *Time of blooming of oat flowers*

Time of blooming \ Year	Number of days			
	1927	1928	1929	Total
a.m. 9.00	0	1	0	1
10.00	0	0	0	0
11.00	0	0	0	0
12.00	0	0	0	0
p.m. 12.45	1	0	0	1
1.00	0	0	0	0
1.15	4	0	0	4
1.30	3	3	3	9
1.45	2	1	0	3
2.00	9	4	4	17
2.15	8	6	6	20
2.30	4	4	5	13
2.45	14	4	5	23
3.00	11	11	7	29
3.15	10	8	4	22
3.30	8	3	5	16
3.45	2	1	0	3
4.00	2	6	1	9
4.15	2	3	1	6
4.30	0	3	2	5
4.45	0	0	1	1
5.00	0	1	1	2
5.15	0	1	2	3
5.30	0	0	0	0
5.45	0	0	1	1
6.00	0	0	0	0
non-blooming days	15	13	15	43
Total	95	73	63	231

Of the results shown in Table 3, those of 1927 have already been reported. The whole number of days on which observation was made was 95, on 15 of which no blooming occurred. The earliest time of blooming on 80 days was between 12 m. and 12:45 p.m. and the latest was 4:15 p.m. The most intense blooming took place between 2 and 3 p.m. Further observations, however, showed that in 1928 no flowers bloomed on 13 days out of the 73 and on the remaining 60 days the

earliest flower opened at 9 a.m. This occurred only once during three years of observations and was an unique case. The next earliest one opened at 1:30 p.m. and the latest one at 5:15 p.m. Then in 1929, within 63 days of observation no blooming occurred on 15 days; the earliest flower during 48 days opened at 1:15 p.m. and the latest at 5:45 p.m. The maximum blooming was between 2 and 3:30 p.m. on most of these days. As shown above, the time of blooming of oat flowers varies much according to the day. These results indicate therefore that of 231 days on which observations were made no flower opened on 43 days.

4. Summary

1. The first flowers to open on oat plants are in the panicle on the primary stem and then those on the other tillering stems.

2. The days required to complete the blooming of all the panicles on a plant vary according to the climatic conditions, but range from 29 to 31 days. When temperature is high less days are required and when low, more days are required.

3. The first flowers to open in each panicle are in the spikelet at the top of the main axis.

4. The days required to complete the blooming of the entire panicle vary according to the external conditions, but under favourable conditions about 8 days are needed.

5. The blooming of oat flowers occurs within a certain period of time of the day which varies according to the climatic conditions. When the conditions are favourable it is shorter and when unfavourable it is longer and ordinarily it is about an hour in length.

6. The writer observed the blooming of oats under natural conditions on 231 days during 1927–1928–1929 and found that on 43 days out of them no blooming took place.

7. The time of blooming varies considerably according to the day, the earliest being 12:45 p.m. and the latest 5:45 p.m.

8. The blooming of oats in natural condition usually occurs in the afternoon, but of the 183 days on which flowers opened there was one day on which blooming took place at 9 a.m.

II. Ecological Studies on the Blooming of Oat Flowers

A. Relation between the blooming of oats and environmental factors

From observations on the blooming of oats, the writer found that there is a marked difference in the time of blooming during the day and also that on a number of days no blooming occurred at all. It can be easily considered that blooming is greatly influenced by the environmental factors. Therefore the writer made investigations on the relation between the environmental factors and blooming.

1. Relation between Blooming and Temperature

(i) Range of Temperature on the Days on which Blooming occurs and of the Temperature most suitable for Blooming

It has been believed that proper temperature is necessary for the blooming of cereals. A temperature higher or lower than certain degress hinders blooming, that is, blooming takes place only within a certain range of temperature. There exists, however, within this range an optimum temperature for the blooming of any cereals. In the present study, the writer has considered two kinds of temperature relations; the highest temperature of the day, and the temperature at the time when blooming occurs, because the blooming of oats takes place after the temperature has fallen from its highest point of the day and therefore that highest temperature plays an important part in the present study.

The highest temperature and the temperature at the time of blooming on each day of observation are shown in the appended tables. The maximum and minimum degrees of these temperatures and the temperature most suitable for blooming are picked out from those tables and are shown in Tables 4, 5 and 6.

As was already reported by the writer,[20] the highest temperature in 1927 was 16.8°–30.0°C. while the temperature at the time of blooming was 14.0°–29.4°C., but as shown in Table 4, during the three years (1927–28–29) of his observations the highes. temperatures lay between 32.8° and 15°C. and the temperatures at the time of blooming were between 29.8°C. and 14°C. As shown in Table 5, the number of days on which the highest temperatures were between 27°C. and 29°C. is the largest, being 30.85% of the whole number of flowering days (188 days),

and the number of days on which the highest temperatures were 24°–26°C. and 21°–23°C. comes next, being 22.34% in the former and 23.94% in the latter.

As shown in Table 6, the number of days on which the temperatures at the time of blooming were between 24°C. and 26°C. is the largest, being 35.11% of all the flowering days, on 21.80% of the days the temperatures were 21°–23°C., on 18.08% of the days they were 18°–20°C. and on 18.62% of the days they were 27°–29°C. According the temperature snitable for blooming may be said to be 27°–29°C. as regards the highest temperature of the day and 24°–26°C. at the time of blooming.

TABLE 4. *Range of the highest temperature and temperature at time of blooming*

	1927	1928	1929
Highest temperature (C.)	30.0°—16.8°	32.8°—19.5°	32.6°—15.0°
Temperature at the time of blooming (C.)	29.4°—14.0°	29.2°—14.4°	29.8°—14.4°

TABLE 5. *Frequency distribution of the highest temperature on blooming days*

The highest temperature (C.)	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	Total
Year	Number of days																		
1927	0	1	2	0	2	5	9	7	13	6	7	5	8	7	7	1	0	0	89
1928	0	0	0	0	2	2	2	3	1	2	8	6	13	10	5	4	1	1	60
1929	1	2	2	1	3	1	5	4	1	2	0	6	3	1	4	3	5	4	48
Total	1	3	4	1	7	8	16	14	15	10	15	17	24	18	16	8	6	5	188
		8			16			45			42			58			19		188
%		4.26			8.51			23.94			22.34			30.85			10.11		100

TABLE 6. *Frequency distribution of temperature at time of blooming*

Temperature at the time of blooming (C.)	14°	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	Total
Year	Number of days																
1927	0	1	3	1	2	4	10	8	10	8	9	3	10	9	2	0	80
1928	0	0	0	0	3	2	2	3	4	1	7	15	11	7	4	1	60
1929	1	1	2	3	4	4	3	2	4	1	3	3	5	3	4	5	48
Total	1	2	5	4	9	10	15	13	18	10	19	21	26	19	10	6	188
	12				34			41			66			35			188
%	6.38				18.08			21.80			35.11			18.62			100

(ii) Relation between Variation of Temperature and Blooming

a. Variation of temperature on blooming days

Temperature undergoes a continuous variation in the course of a day. The manner of its variation varies according to days and an exceedingly complicated variation can be experienced. Generally temperature is low at sunrise, then gradually it rises and the highest point is reached at about two o'clock in the afternoon and then it falls little by little.

The writer observed the variation of temperature at every 15 minute interval from 6 a.m. to 6 p.m. every day during his entire observations. He observed the variation of temperature on each of these days and also the flowering time of oats. These results are shown in the appended tables. The writer ascertained that there is an intimate relation between the variation of temperature and blooming and that blooming of oats occurs while the day's temperature is falling from its highest point. Scarcely any blooming takes place while the temperature is rising. Oats differ greatly in this respect from the flowering of many other cereals. The difference between the highest temperature and that at 8 a.m. and between the highest temperature and that at the time of blooming, and also the interval from the time of highest temperature to that when blooming begins to occur are shown in Tables 7, 8 and 9. As shown in Table 7, the difference between the highest temperature and that at 8 a.m. is 3°–5°C. in most cases and out of the 188 days of observation the same results were obtained on 99

TABLE 7. *Variation of difference between the highest temperature and the temperature at 8 a.m. on blooming days*

Difference of temperature (C.)	0°	1°	2°	3°	4°	5°	6°	7°	8°	9°	Total
Year					Number of days						
1927	1	4	7	14	12	14	9	11	4	4	80
1928	1	8	8	14	10	8	6	2	2	1	60
1929	0	2	6	6	9	12	7	2	3	1	48
Total	2	14	21	34	31	34	22	15	9	6	188
		37			99			46		6	188
%		19.68			52.66			24.47		3.19	100

TABLE 8. *Variation of difference between the highest temperature and the temperature at time of blooming*

Difference of temperature (C.)	Number of days 1927	1928	1929	Total
0.2°—0.5°	9	7	5	21
0.6—1.0	28	11	8	47
1.1—1.5	11	11	4	26
1.6—2.0	20	12	9	41
2.1—2.5	4	8	7	19
2.6—3.0	4	3	6	13
3.1—3.5	3	3	2	8
3.6—4.0	1	2	3	6
4.1—4.5	0	2	0	2
4.6—5.0	0	1	2	3
5.1—5.5	0	0	0	0
5.6—6.0	0	0	1	1
6.1—6.5	0	0	0	0
6.6—7.0	0	0	0	0
7.1—7.5	0	0	1	1
Total	**80**	**60**	**48**	**188**

days. There are, however, cases in which the difference in temperature is less than 2°C., but even then when compared with that before 8 a.m. a greater difference appears.

The difference between the highest temperature and that at the time of blooming (Table 8) is not uniform each day, the least being 0.2°C and the greatest 5°C. In a majority of cases the difference is from 0.6° to 2°C. The interval from the time of the highest temperature to that when blooming begins to occur, as shown in Table 9, varies each day. It requires from 30 minutes up to 4 hours, but in most cases the time required is from 1 to 2 hours.

TABLE 9. *Variation of intervals between the time of the highest temperature and that of blooming*

Year	1927	1928	1929	Total		%
The time elapsed (minutes)	Number of days					
15	1	0	0	1	38	20.21
30	6	2	1	9		
45	5	2	5	12		
60	9	6	1	16		
75	6	4	4	14	56	29.79
90	11	3	0	14		
105	5	3	4	12		
120	8	4	4	16		
135	6	4	4	14	49	26.06
150	4	3	5	12		
165	6	3	3	12		
180	7	3	1	11		
195	0	0	4	4	26	13.83
210	2	6	1	9		
225	3	6	1	10		
240	0	2	1	3		
255	0	4	2	6	19	10.11
270	0	1	3	4		
285	1	2	0	3		
300	0	2	4	6		
Total	80	60	48	188	188	100.00

b. Variation in temperature on days when no blooming takes place

The total number of days on which observations were made by the writer in the three years (1927–28–29) was 231 and on 43 days out of this number no blooming took place. On 10 days out of 43 the temperature was low and was out of the range of the highest temperatures of the days on which blooming took place. On the rest of the days it was within the range of the highest temperature of the day on which blooming occurred, but blooming did not take place. Of these days the highest temperature was 24.3°C. on August 31, 1928. But on a number of blooming days it was below that point as shown in Table 10. From the fact that the flower did not bloom even when the highest temperature of the day was within the range of the highest temperature of the blooming days, it may be considered that blooming is caused not only by the highest temperature, but by the influence of the variations of temperature before and after the time of the highest temperature or by other factors. The writer, therefore, investigated the variations in temperature on the days when no blooming occurred. The degrees of the fall of temperature after the time of the highest temperature and the difference between the highest temperature and that at 8 a.m. in the morning are shown in Table 10. The degree of the fall of temperature is indicated by the average of the difference between the highest temperature and the tempratures at the first the second and the third hour after the highest temperature.

As shown in Table 10, in the three years of observations the average fall in degrees during three honrs after the highest temperature was 0.97°C., which was much less when compared with 1.37°C., averaged difference of temperature on blooming days. In some particular days the highest temperature was maintained till the evening or on other days it was observed towards evening. Next the variation during the forenoon, as shown in Table 10, differs on each day or in each year, but on an average, its difference is 2.71°C. when it is low and 3.88°C. when high, the average being 3.40°C. which is less than 5.55°C. the average difference of temperature on the days when blooming took place. From the above mentioned facts it will be seen that the variation of temperature on the non-blooming days is generally very slight when compared with that on blooming days.

TABLE 10. *Comparison of changes of temperature on blooming days on which the temperature is low and those on non-blooming days*

Non-blooming days								
Date	Highest temperature (C.)	Time of the highest temperature	Difference between the highest temperature and that at the 1st, 2nd and 3rd hours after the time of highest temperature				Temperature at 8 a.m. (C.)	Difference between the highest temperature and that at 8 a.m.
			1st hour	2nd hour	3rd hour	Average		
1927								
July 18	21.7°	1.30 p.m.	0.3°	0.7°	−0.1°	0.3°	19.8°	0.9°
Aug. 24	20.2	1.45 p.m.	0.7	0.7	0.4	0.6	18.4	1.6
„ 30	19.8	1.15 p.m.	0.2	0.6	0.4	0.4	19.2	0.6
Sep. 3	17.5	4.45 p.m.	—	—	—	—	16.2	1.3
„ 10	20.2	11.30 a.m.	0.3	1.0	1.4	0.9	17.8	3.0
„ 17	20.0	2.00 p.m.	0.8	2.6	3.7	2.4	18.4	1.6
Oct. 2	20.2	1.00 p.m.	0.4	0.8	2.0	1.1	18.6	1.6
„ 9	19.5	2.15 p.m.	0.7	1.1	—	0.9	15.0	4.5
„ 11	16.4	2.00 p.m.	0.6	3.0	—	1.8	9.8	6.6
„ 14	15.0	1.15 p.m.	1.0	0.2	3.0	1.4	9.6	5.4
Average	19.05					1.09	16.28	2.71
1928								
Aug. 31	24.3	1.45 p.m.	0.3	0.5	0.8	0.5	22.5	1.8
Sep. 1	22.1	1.00 p.m.	0.5	1.1	1.4	1.1	19.1	3.0
„ 12	23.5	12.00 m.	1.1	2.0	2.0	1.8	19.1	4.4
„ 19	18.1	9.00 a.m.	0.9	1.7	2.1	1.6	16.3	1.8
„ 21	20.5	1.00 p.m.	0.0	1.0	1.0	0.7	19.1	1.5
„ 23	16.3	12.00 m.	0.1	0.8	1.0	0.6	12.8	3.5
„ 26	18.5	11.00 a.m.	1.3	1.0	1.0	1.1	13.6	4.9
„ 28	19.0	3.30 p.m.	—	—	—	—	16.0	3.0
Oct. 1	16.5	1.00 p.m.	0.3	0.8	1.5	0.9	12.0	4.5
„ 3	19.6	3.45 p.m.	—	—	—	—	14.5	5.1
„ 5	16.3	1.15 p.m.	0.3	0.3	0.8	0.5	9.2	7.1
Average	19.52					0.98	15.84	3.69
1929								
July 4	20.2	3.00 p.m.	0.6	0.0	0.8	0.5	17.6	2.6
„ 5	16.6	12.30 p.m.	0.6	0.7	0.5	0.6	15.6	1.0
„ 6	17.1	1.00 p.m.	0.3	0.5	0.5	0.4	16.1	1.0
Aug. 4	23.6	4.00 p.m.	2.2	3.0	—	2.6	19.4	4.2
„ 22	18.4	1.30 p.m.	0.2	0.8	1.4	0.8	14.1	4.3
Sep. 30	15.1	1.00 p.m.	0.4	0.7	—	0.6	11.9	5.1
Oct. 7	19.2	1.45 p.m.	0.3	0.1	0.6	0.3	14.5	4.7
„ 8	18.0	1.30 p.m.	—	4.6	—	—	16.6	1.4
„ 10	15.0	1.15 p.m.	0.5	0.8	—	0.6	10.6	4.4
„ 11	15.1	9.00 a.m.	0.9	1.3	1.9	1.4	14.0	1.1
„ 17	19.6	2.00 p.m.	0.4	1.6	—	1.0	8.2	11.4
„ 18	15.1	1.00 p.m.	0.2	0.6	—	0.4	9.8	5.3
Average	17.75					0.84	14.08	3.88
Av. of three years						0.97	15.38	3.40

TABLE 10. (*Continued*)

Blooming days									
Date	Highest temperature (C.)	Time of the highest temperature	Difference between the highest temperature and that at the 1st, 2nd and 3rd hours after the time of highest temperature				Temperature at 8 a.m. (C.)	Difference between the highest temperature and that at 8 a.m.
			1st hour	2nd hour	3rd hour	Average		
1927								
Sep. 13	21.0°	1.45 p.m.	0.5°	1.0°	1.2°	0.9°	15.6°	5.4°
„ 21	20.0	10.30 a.m.	0.2	1.6	2.2	1.3	16.2	3.8
„ 22	19.6	11.30 a.m.	0.8	0.8	1.4	1.0	13.4	6.2
„ 23	20.8	2.00 p.m.	0.6	1.8	2.0	1.5	11.6	9.8
„ 24	20.6	1.15 p.m.	0.4	1.2	1.8	1.1	12.2	8.4
„ 25	19.4	1.45 p.m.	0.6	1.0	3.0	1.5	11.6	7.8
„ 26	21.0	2.00 p.m.	1.1	1.4	3.0	1.8	12.4	8.6
„ 27	21.4	2.15 p.m.	0.2	1.2	3.0	1.5	16.6	4.8
Oct, 10	20.4	10.45 a.m.	0.6	1.0	3.2	1.6	15.4	5.0
„ 16	16.4	1.45 p.m.	2.0	4.4	5.4	3.9	7.4	9.0
Average	20.06					1.61	13.21	6.88
1928								
July 16	22.5	10.30 a.m.	0.3	0.4	0.5	0.4	21.5	1.0
„ 19	23.5	1.15 p.m.	1.0	1.0	1.6	1.2	20.0	3.3
Sep. 16	22.0	1.00 p.m.	0.8	0.4	1.2	0.8	18.5	1.5
„ 17	19.5	11.00 a.m.	0.5	0.2	0.8	0.5	17.5	2.0
„ 18	20.2	1.00 p.m.	0.6	1.5	1.6	1.2	13.4	6.8
„ 20	24.5	1.30 p.m.	0.5	2.5	2.5	1.8	18.5	6.0
„ 22	19.8	1.15 p.m.	1.6	1.1	2.1	1.6	13.5	6.3
„ 24	21.0	1.30 p.m.	0.5	1.0	1.7	1.0	14.5	5.5
„ 27	20.0	10.30 a.m.	0.6	0.5	0.5	0.5	19.0	1.0
„ 29	22.0	11.00 a.m.	0.0	3.3	1.2	1.5	17.0	5.0
Oct. 2	21.5	10.30 a.m.	0.5	1.1	0.5	0.7	18.0	5.5
Average	21.50					1.02	17.40	4.08
1929								
July 8	19.4	12.00 m.	1.5	0.6	0.9	1.0	14.7	5.3
Sep. 21	22.6	10.00 a.m.	1.0	2.4	5.4	2.9	16.8	5.8
„ 23	19.8	1.15 p.m.	0.6	1.4	2.3	1.4	12.0	7.8
„ 24	21.5	12.30 p.m.	0.5	0.9	1.3	0.9	16.4	5.0
„ 26	21.0	1.00 p.m.	0.8	1.4	2.5	1.6	16.0	5.0
„ 27	19.4	1.30 p.m.	0.9	1.4	—	1.1	15.4	4.0
„ 29	21.4	12.00 m.	1.8	3.0	4.0	2.9	17.6	3.8
Oct. 1	16.8	1.45 p.m.	0.2	0.8	1.9	1.0	10.0	6.8
„ 3	22.1	2.15 p.m.	1.3	2.7	5.0	3.0	12.2	9.9
„ 5	21.6	2.15 p.m.	0.6	1.3	3.0	1.6	16.6	5.0
„ 9	17.5	12.00 m.	1.3	0.4	0.7	0.8	12.4	5.5
„ 16	15.0	12.00 m.	0.6	0.4	1.2	0.7	10.4	4.6
Average	19.84					1.49	14.21	5.71
Av. of three years						1.37	14.94	5.55

(iii) Relation between Variation of Temperature and the Time of Blooming

The time of the day's highest temperature in Sapporo, according to the writer's observations, was generally reached at about 1 or 2 o'clock when the weather was clear and fine, but the time depends upon the daily condition of the weather. On the other hand, as related (in I. 3), the most intense blooming took place at about 2 or 3 o'clock in the afternoon, but there was a marked difference in the time of blooming according to the day. Blooming, as is shown above, takes place when the temperature begins to fall after having passed its highest degree. Therefore it is possible to think that there may exist some close relation between the time of the highest temperature and that of blooming. Regarding this the writer in his preliminary report[20] stated that the sooner the temperature reaches its highest point, the quicker comes the time of blooming. The results of the writer's further observations rendered certain this fact. However when temperature does not begin to fall soon after it has reached its highest degree, but remains stationary for a considerable period, the time of blooming is delayed even when the time of the highest temperature is early. The writer observed such cases often on the hottest summer days, July 27, 28 and August 7, 1929 (appended table 7). Generally on the days when the highest temperature is reached comparatively early, it required much time before blooming occurs, while on the days when the highest temperature is reached later, there is a tendency for the flowers to bloom shortly after the temperature begins to decline.

2. *Relation between Blooming and Humidity*

(i) Range of Humidity on Blooming Days and Humidity suitable for Blooming

The writer in the present study has studied humidity in two different aspects, one is the lowest humidity of the day and the other, humidity at flowering time. From the results of his observations as shown in the appended tables, the writer determined the range of the lowest humidity on blooming days, the range of humidity at the time of flowering and the humidity suitable for blooming, which are shown in Tables 11, 12 and 13 respectively. The lowest humidity on a blooming day (Table 11) was 37.7 in 1929 and the highest one was 91 in the same

year, the lowest humidity at the time of blooming was 41.5 in 1929 and the highest one was 100 in 1928. As shown in Table 12, the days on which the lowest humidity was 60 were the most numerous, being 38.30% of all the flowering days (188); this is followed by the days on which the lowest percentage was 50, and then the days on which the lowest percentage was 70. Further, the number of days on which the humidity at the time of blooming, as shown in Table 13, was 70% was the largest, being 36.70% of the total, and this is followed by the number of those days on which the humidity was 60%.

TABLE 11. *Range of the lowest humidity on blooming days and that of humidity at the time of blooming*

Year	Lowest humidity on blooming days		Humidity at the time of blooming	
	Minimum	Maximum	Minimum	Maximum
	%	%	%	%
1927	49.0	86.0	53.0	95.0
1928	39.5	90.0	44.5	100.0
1929	37.7	91.0	41.5	99.0

TABLE 12. *Frequency distribution of the lowest humidity on blooming days*

	Lowest humidity / Year	30%	40%	50%	60%	70%	80%	90%	100%	Total
Number of days	1927	0	1	10	41	21	7	0	0	80
	1928	1	8	21	19	8	2	1	0	60
	1929	1	6	22	12	5	1	1	0	48
	Total	2	15	53	72	34	10	2	0	188
	%	1.06	7.98	28.19	38.30	18.09	5.32	1.06	0	100

TABLE 13. *Frequency distribution of humidity at the time of blooming in 1927, 1928 and 1929*

	Humidity of blooming time / Year	30%	40%	50%	60%	70%	80%	90%	100%	Total
Number of days	1927	0	0	2	18	43	12	5	0	80
	1928	0	5	7	22	16	7	3	0	60
	1929	0	1	12	17	10	4	3	1	48
	Total	0	6	21	57	69	23	11	1	188
	%		3.19	11.71	30.32	36.70	12.23	5.85	0.53	100

(ii) Relation between Variation in Humidity and Blooming

The blooming of oat flowers occurs within the range of humidity as stated above (2.i). The humidity of the day constantly changes with the changes of temperature and therefore it can be considered that there exists a close relation between the changes of humidity and blooming. The writer studied this relation from the results of his observations which are shown in the appended tables.

Variations of humidity on blooming days

The writer in order to know the variations in humidity on blooming days observed the difference between the humidity at 8 a.m. and the lowest humidity, and the difference between the lowest humidity and the humidity at the time of blooming and the interval between the time of the lowest humidity and the flowering time. The data are arranged in Tables 14, 15 and 16. The number of days on which the difference between the humidity at 8 a.m. and the lowest humidity was 11–20% was the largest; next to this comes the number of days on which the difference was 21–25% and this is followed by the number of days on which the difference was 6–10%. The number of days on which the difference between the lowest humidity and the humidity at the flowering time in the entire day was 3–7% was the largest. Lastly the days on which from two to three hours elapsed from the time of the lowest humidity to the flowering time was the largest in number.

TABLE 14. *Variation of difference between the lowest humidity and humidity at 8 a.m. on the blooming days*

Year	1927	1928	1929	Total		%
Difference of Humidity (%)	Number of days					
1	1	0	0	1		
2	0	0	0	0		
3	0	0	0	0	7	3.72
4	1	0	1	2		
5	2	1	1	4		
6	1	0	2	3		
7	3	0	1	4		
8	2	1	1	4	19	10.11
9	4	0	2	6		
10	0	1	1	2		
11	3	0	3	6		
12	5	1	1	7		
13	3	1	3	7	30	15.96
14	4	0	0	4		
15	1	2	3	6		
16	4	1	3	8		
17	2	2	2	6		
18	3	2	1	6	39	20.74
19	5	4	2	11		
20	4	1	3	8		
21	2	3	3	8		
22	3	2	1	6		
23	5	2	3	10	40	21.28
24	5	2	2	9		
25	2	3	2	7		
26	1	2	0	3		
27	0	5	2	7		
28	3	2	0	5	25	13.30
29	3	2	1	6		
30	2	2	0	4		
31	0	0	1	1		
32	2	0	0	2		
33	1	3	1	5	13	6.91
34	0	3	0	3		
35	1	1	0	2		
36	0	1	0	1		
37	0	2	0	2		
38	1	4	1	6	15	7.98
39	0	0	0	0		
40	1	4	1	6		
Total	80	60	48	188	188	100.00

TABLE 15. *Variation of difference between the lowest humidity and the humidity at the time of blooming*

Year	1927	1928	1929	Total		%
Difference of humidity (%)	Number of days					
0	5	0	1	6	6	3.19
1	4	4	1	9	73	38.83
2	4	5	4	13		
3	10	3	6	19		
4	11	3	2	18		
5	3	7	4	14		
6	7	3	4	14	67	35.64
7	8	4	3	15		
8	5	8	2	15		
9	5	1	3	9		
10	6	5	3	14		
11	2	1	0	3	23	12.23
12	2	0	4	6		
13	1	3	1	5		
14	2	0	3	5		
15	0	2	2	4		
16	1	5	0	6	14	7.45
17	1	0	0	1		
18	2	1	0	3		
19	0	1	2	3		
20	0	1	0	1		
21	0	0	1	1	2	1.06
22	0	0	1	1		
23	0	0	0	0		
24	0	0	0	0		
25	0	0	0	0		
26	0	1	0	1	3	1.60
27	1	0	0	1		
51	0	0	1	1		
Total	80	60	48	188		100.00

TABLE 16. *Variation of interval between the time of the lowest humidity and blooming time*

Year	1927	1928	1929	Total		%
Length of time (minutes)	Number of days					
15	2	1	0	3	30	15.96
30	4	3	3	10		
45	3	3	1	7		
60	5	2	3	10		
75	3	3	5	11	50	26.60
90	3	7	1	11		
105	5	4	2	11		
120	7	4	6	17		
135	8	4	6	18	46	24.47
150	6	4	4	14		
165	2	2	3	7		
180	4	2	1	7		
195	4	4	1	9	40	21.28
210	3	6	1	10		
225	6	3	0	9		
240	7	4	1	12		
255	0	1	3	4	17	9.04
270	1	1	3	5		
285	3	1	1	5		
300	2	1	0	3		
315	0	0	1	1	3	1.60
330	0	0	0	0		
345	1	0	0	1		
360	1	0	0	1		
375	0	0	0	0	2	1.06
390	0	0	2	2		
Total	80	60	48	188		100.00

Variations in humidity on non-blooming days

As has already been mentioned, there were a number of days during the period of observations on which no blooming occurred at all. The variations in humidity on each of these days are shown by the difference between the humidity at 8 a.m. and the lowest humidity, and the

TABLE 17. *Comparison between changes of humidity on blooming days and those on non-blooming days*

Non-blooming days									
Date	Lowest humidity	Time of lowest humidity	Difference between the lowest humidity and those at the 1st, 2nd and 3rd hours after the time of the lowest %				Humidity at 8 a.m.	Difference between the lowest humidity and that at 8 a.m.
			1st hour	2nd hour	3rd hour	Average		
1927	%		%	%	%	%	%	%
July 18	95	11.15 a.m.	0	0	0	0	95	0
Aug. 24	89	8.30 a.m.	11	11	11	11	94	5
„ 30	94	2.30 p.m.	0	0	1	0.3	100	6
Sep. 3	65	1.00 p.m.	5	8	12	8.3	88	23
„ 10	85	11.45 a.m.	0	9	9	6.0	89	13
„ 17	75	1.45 p.m.	5	4	8	5.7	84	9
Oct. 2	75	8.45 a.m.	6	5	5	5.3	80	5
„ 9	70	12.15 p.m.	0	5	14	6.3	94	24
„ 11	58	2.00 p.m.	5	8	—	6.5	80	22
„ 14	63	1.30 p.m.	0	1	2	1.0	80	17
Average	76.60					5.04	80.40	12.4
1928								
Aug. 31	95	10.00 a.m.	1	1	2	1.3	98	3
Sep. 1	89	1.00 p.m.	5	9	10	8.0	99	10
„ 12	64	12.00 m.	3	11	13	9.0	91	27
„ 19	70	9.00 a.m.	6	17	11	10.1	82	12
„ 21	62	1.15 p.m.	2	−5	6	1.0	74	12
„ 23	87	1.15 p.m.	7	3	8	6.0	87	0
„ 26	50	11.00 a.m.	7	5	5	5.6	62	12
„ 28	62	11.00 a.m.	2	2	5	3.0	70	8
Oct. 1	43	2.30 p.m.	4	12	16	8.0	72	29
„ 3	56	10.00 a.m.	0	20	16	12.0	78	22
„ 5	43	1.15 p.m.	12	−7	—	2.5	72	29
Average	65.55					6.04	80.45	14.9
1929								
July 4	74	12.00 m.	11	11	7	9.6	86	12
„ 5	86	12.30 p.m.	4	6	−5	1.6	98	12
„ 6	91	12.30 p.m.	1	2	3	2.0	95	4
Aug. 4	71	2.00 p.m.	1	4	25	10.0	87	16
„ 22	43	2.15 p.m.	4	6	7	5.6	55	12
Sep. 30	55	1.45 p.m.	3	—	10	6.5	77	12
Oct. 7	71	2.30 p.m.	3	7	—	5.0	95	24
„ 8	82	1.00 p.m.	0	15	16	10.3	98	14
„ 10	61	1.15 p.m.	6	16	19	10.3	78	17
„ 11	57	10.00 a.m.	3	7	3	4.3	62	5
„ 17	60	2.30 p.m.	0	3	—	1.5	100	40
„ 18	61	2.00 p.m.	5	—	—	5.0	83	22
Average	67.67					5.98	84.50	15.8
Av. of three years						5.35	84.45	14.37

TABLE 17. (*Continued*)

Date	Lowest humidity	Time of lowest *humidity*	Difference between the lowest humidity and those at the 1st, 2nd *and 3rd hours after the* time of the lowest %				Humidity at 8 a.m.	Difference between *the lowest* humidity *and that* at 8 a.m.
			1st hour	2nd *hour*	3rd hour	Aver age		
Blooming days								
1927	%		%	%	%	%	%	%
Sep. 13	71	11.15 a.m.	1	1	1	1.0	88	17
„ 21	60	10.00 a.m.	1	7	10	6.0	69	9
„ 22	58	11.30 a.m.	12	12	12	12.0	82	24
„ 23	64	1.30 p.m.	0	6	5	3.4	87	23
„ 24	55	12.30 p.m.	9	13	9	10.3	87	32
„ 25	63	12.45 p.m.	8	8	8	8.0	87	24
„ 26	64	1.30 p.m.	0	12	16	9.3	87	23
„ 27	58	12.15 p.m.	7	10	6	7.6	78	20
Oct. 10	54	12.45 p.m.	12	7	6	8.3	73	19
„ 16	61	11.45 a.m.	13	13	11	12.3	78	17
Average	60.08					7.82	81.60	20.80
1928								
July 16	92	11.00 a.m.	5	4	3	4.0	95	3
„ 19	60	12.30 p.m.	3	6	10	6.3	80	20
Sep. 16	55	12.30 p.m.	14	15	16	15.0	84	29
„ 17	56	1.15 p.m.	3	3	3	3.0	66	10
„ 18	52	2.15 p.m.	1	8	10	6.3	82	30
„ 20	63	1.15 p.m.	5	3	5	4.3	84	21
„ 22	53	1.15 p.m.	0	11	20	10.3	87	32
„ 24	75	1.00 p.m.	5	6	19	10.0	100	25
„ 27	40	2.30 p.m.	1	3	7	3.9	55	15
„ 29	49	3.15 p.m.	3	—	—	3.0	87	38
Oct. 2	52	12.00 m.	3	2	10	5.0	57	5
Average	58.82					6.46	79.73	20.73
1929								
July 8	74	12.00 m.	5	0	−2	1.0	97	23
Sep. 21	42	10.30 a.m.	10	9	51	23.0	65	23
„ 23	57	1.15 p.m.	1	4	4	3.3	77	20
„ 24	51	1.00 p.m.	2	6	9	5.7	67	26
„ 26	53	1.15 p.m.	4	11	24	13.0	74	21
„ 27	52	1.00 p.m.	3	2	5	3.3	76	24
„ 29	67	12.00 m.	12	7	13	10.7	88	21
Oct. 1	45	1.00 p.m.	6	12	14	10.6	86	41
„ 3	52	2.00 p.m.	−3	12	26	11.7	77	25
„ 5	63	2.15 p.m.	3	16	24	14.3	64	1
„ 9	53	2.00 p.m.	9	10	28	15.6	62	29
„ 16	58	12.00 m.	—	3	1	1.0	72	14
Average	55.59					9.43	77.08	22.33
Av. of three years						7.90	79.47	21.29

humidity at one, two and three hours thereafter. By comparing these with the variations on blooming days the writer obtained the results shown in Table 17. On non-blooming days during three years the average difference between the lowest humidity and that at 8 a.m. was 14.37% and the increase in humidity after the time of the lowest humidity 5.35%, but on blooming days the former was 21.29% and the latter 7.90%.

(iii) Relation between Variation in Humidity and Blooming Time

When the variations of humidity in the course of the day are considered, it can be said that blooming always begins to take place while humidity is increasing from the lowest percentage and in the majority of cases blooming occurs 1–2 hours after the time of the lowest humidity. Accordingly there exists an intimate relation between the time of the lowest humidity and that of blooming; that is to say, on the days when the time of the lowest humidity comes early, the time of blooming is also early, and when the former comes late, the latter too is late.

3. *Relation between Blooming and Rainfall*

The data as to the relation between blooming and rainfall which were observed by the writer is shown in Table 18. There were 15 rainy days in 1927, 9 in 1928 and 10 in 1929, the total being 34 days, and these rainy days can be divided into the days on which there were showers and the days on which it rained the whole day. Blooming occurred as usual, in spite of rainfall, on those days when rain began to fall as early as about noon or as late as two o'clock in the afternoon. Rain shows a tendency to accelerate blooming. But on the days when it rained the whole day in some cases blooming occurred and in others no blooming took place. According to the writer's observations in 1927, it rained the whole day on July 7 and 21, and on September 4 and 6, but blooming occurred, which on July 18 and 24 and on September 3, 10 and 17 no blooming occurred under similar conditions. The reason for this can be understood by considering the temperature on these days. On flowering days temperature is generally high and on the days when temperature is high blooming takes place even when it rains, because there are considerable changes in temperature on these days. On the days when no blooming occurred, temperature was low, and changes in temperature were very slight.

4. *Relation between Blooming and Wind*

The writer, in order to ascertain the relation between blooming and wind, compared the number of flowers opened and the time of blooming on days when the average velocity of wind from 6 a.m. to 6 p.m. was 4.42–5.77 m/s with those on days when the average velocity from 6 a.m. to 6 p.m. was 1.14–2.91 m/s. The results are shown in Table 19.

Observations were made on blooming and the time of blooming on 11 days when the average velocity of wind was 4.42–5.77 m/s and 12 days when the average velocity of wind was 1.14–2.91 m/s. In the former it was found that blooming did not occur on July 18 and October 2 out of the 11 days and in the latter blooming occurred without exception. The blooming time of the day was between 2 and 4 o'clock in the afternoon on both days, and no difference between them was observed.

Thus the force of wind does not effect blooming nor the time of blooming in any way whatever.

TABLE 18. *Comparison between the amount of rainfall on non-blooming days and that on blooming days*

Non-blooming days												
Time / Date	6–7 a.m.	7–8 a.m.	8–9 a.m.	9–10 a.m.	10–11 a.m.	11–12 a.m.	12–1 p.m.	1–2 p.m.	2–3 p.m.	3–4 p.m.	4–5 p.m.	5–6 p.m.
	amount of rainfall (m.m.)											
1927												
July 18	0.1	2.2	4.5	0.1	3.8	5.8	4.4	1.0	0.6	0.2	0.1	0.5
Aug. 24	3.2	8.8	4.1	7.9	8.0	1.5	0.1	0.0	—	0.3	0.2	0.2
,, 30	0.4	2.1	6.5	6.1	5.4	6.4	2.4	0.4	0.0	—	—	0.0
Sep. 3	0.0	0.1	0.0	0.0	0.2	1.9	2.4	2.4	3.2	0.2	—	—
,, 10		—	0.0	0.2	0.0	0.0	0.0	0.4	0.9	0.3	0.1	0.1
,, 17		0.2	0.9	0.2	0.0	0.0						
1928												
Aug. 31	0.0	0.2	0.4	1.4	2.3	2.6	5.5	1.8	5.1	3.5	3.4	3.9
Sep. 1	1.8	0.8	0.1	0.0	0.0	—	—	0.0	0.1	0.6	1.3	0.3
,, 19				0.0	0.8	0.4	0.1	0.8	0.7	0.8	1.4	1.0
,, 23			0.0	0.0	0.4	0.5	0.7	1.2	1.0	1.3	1.9	2.0
1929												
Oct. 8	0.3	0.2	0.1	0.1	0.0	0.3	0.1	0.1	4.3	3.7	1.4	0.9

Table 18. (*Continued*)

Blooming days Time / Date	6–7 a.m.	7–8 a.m.	8–9 a.m.	9–10 a.m.	10–11 a.m.	11–12 a.m.	12–1 p.m.	1–2 p.m.	2–3 p.m.	3–4 p.m.	4–5 p.m.	5–6 p.m.
				amount of rainfall (m.m.)								
1927												
July 13						0.0*	0.4	2.1 **54†**	2.0 **39**	3.5	10.7	12.5
„ 16									0.3 **52**	0.0 **64**	0.0 **7**	-
„ 17	0.2	0.1	0.0	0.2	0.1	0.3	0.0	0.8 **9**	0.0 **90**	0.0 **17**	0.0	0.0
„ 21	0.2				0.0	0.0	0.1	0.3	0.8 **45**	0.6	0.4	1.3
Aug. 3					0.6	2.9				**19**	13.1 **5**	
Sep. 4		0.0	0.2		0.1	0.1	0.2	0.0	0.1 **48**	1.0 **16**		0.2
„ 16				0.0	0.0	0.1	0.0	0.0	**59**	**23**	0.4	0.0
„ 18	0.0	0.2	0.0				0.0	0.0	0.1 **56**	**94**	**8**	
„ 19						0.0	0.0	0.2	2.0	3.3 **165**	1.2 **21**	0.3
1928												
July 16	0.4	0.5	0.8	0.9	0.4	9.1	3.2	3.1	3.4 **11**	0.1 **5**		
„ 17							0.0	0.1 **260**	**386**	1.6 **13**	0.1	0.0
„ 30			0.0	0.0		0.0	0.0	0.0 **3**	**26**	**40**	**8**	
Sep. 13	0.0				0.0	2.2	3.6	1.0 **3**	2.4 **9**	1.0	0.2	1.1
„ 16								0.1	0.0 **1**	0.0	0.0	0.0
1929												
July 14	0.0	0.2	0.4	0.6	1.1	0.0			0.0 **81**	0.0 **78**	0.0 **4**	0.0
„ 23	0.0	0.0		0.0	1.4	0.8	1.0	0.5 32	0.5 **14**	4.2 **2**	3.5	3.7
„ 25									0.7	2.7 **138**	**31**	0.0
Aug. 14										0.1 **44**	0.0 **9**	0.0
„ 24			0.0	0.2	0.0		0.0	1.3	0.4	0.0 **22**	0.0 **1**	0.0
„ 27	3.7	1.3	0.5	0.2	0.0	0.0	1.1	0.0	**2**	0.0 **23**		
„ 28							0.0	0.2		0.2 **39**	0.0	
„ 29								1.3	4.0 **10**	2.0 **2**	1.5	0.6

* ....amount of rainfall

† ....number of flowers bloomed

TABLE 19. *Number of blooming flowers days when the average velocity of wind from 6.00 a.m. to 6.00 p.m. was 4.42–5.77 m/s and that on days when the average velocity of wind from 6.00 a.m. to 6.00 p.m. was 1.14–2.91 m/s.*

Date	Average velocity of wind from 6 a.m. to 6 p.m.	Blooming time															Total
		2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	
		Number of blooming flowers															
July 26	5.77							8	26	9	12						55
Aug. 29	5.58			2	84	30	27	10	2	1							154
Sep. 2	4.48			1	0	0	4	2	8	4	1						20
„ 9	4.42				11	13	1	1									26
„ 18	4.95				56	9	44	23	18	8							158
„ 27	4.51				16	24	36	15	5								96
„ 29	4.42				1	9	7	10	16	7	3						53
Oct. 2	7.06																0
„ 8	5.42					27	18	11	3								59
Average	5.18																
July 24	2.16		4	0	9	27	2	22	4	1							69
Aug. 19	1.73									16	22	61	61	16	6		182
„ 20	1.25			18	51	40	12	12	5	2							140
„ 23	1.71	1	0	0	74	54	17	26	22	4							198
Sep. 8	1.25							7	4	4							15
„ 13	1.14						5	4	1	1							11
„ 22	2.18			35	31	24	20	13	6	3							132
„ 24	2.91					10	20	28	17	2							77
Oct. 1	2.17					31	27	27	20	6							111
Average	1.83																

5. *Relation between Blooming and Duration of Sunshine*

In order to understand the relation between blooming and the duration of sunshine the writer compared the hours of sunshine on 10 blooming days with those on 18 non-blooming days. The results are shown in Table 20. Most of the non-blooming days were cloudy or rainy, but on September 17, 1927, September 19 and 21, 1928, and August 22, 1929, the duration of sunshine was considerably long, but no blooming took place. On the other hand, there was sunshine on most

TABLE 20. *Duration of sunshine on blooming days and that on non-blooming days*

Non-blooming days

Date \ Time	6–7 a.m.	7–8 a.m.	8–9 a.m.	9–10 a.m.	10–11 a.m.	11–12 a.m.	12–1 p.m.	1–2 p.m.	2–3 p.m.	3–4 p.m.	4–5 p.m.	5–6 p.m.	Total
				Hours of sunshine (Jordan's recorder)									
1927													
July 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aug. 24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sep. 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.55
„ 10	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
„ 17	0.18	0.10	0.20	0.00	0.00	0.06	0.00	0.30	1.80	0.65	0.00	0.00	3.29
Oct. 3	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.20	0.03	0.00	0.00	0.00	0.26
1928													
Sep. 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 19	0.00	0.00	0.00	0.00	0.20	0.82	0.52	0.82	0.17	0.60	0.65	0.00	3.78
„ 21	0.00	0.70	0.20	0.00	0.50	0.00	0.20	0.90	0.08	0.35	0.25	0.00	3.18
„ 23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 28	0.00	0.00	0.00	0.00	0.40	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.70
1929													
July 4	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18
Aug. 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.10
„ 22	0.07	1.00	1.00	0.72	0.22	0.60	0.40	0.30	0.00	0.60	0.16	0.00	4.71
Oct. 7	0.00	0.00	0 00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 20. (*Continued*)

Blooming days

Date \ Time	6–7 a.m.	7–8 a.m.	8–9 a.m.	9–10 a.m.	10–11 a.m.	11–12 a.m.	12–1 p.m.	1–2 p.m.	2–3 p.m.	3–4 p.m.	4–5 p.m.	5–6 p.m.	Total
1927													
July 13	0.45	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.95
„ 17	00.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 21	0.00	0.45	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55
Aug. 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1928													
July 16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	00.0	0.00	1.96	0.42	0.00	2.38
Aug. 10	0.82	0.68	0.53	0.30	0.00	0.00	0.00	.35	0.00	0.00	0.36	0.20	3.24
„ 24	0.00	0.00	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22
Sep. 13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1929													
July 14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
„ 23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

of the blooming days, but on ten days as shown in Table 20, blooming occurred although the duration of sunshine was short. These results show that there is no intimate relation between the duration of sunshine and blooming.

6. *Relation between Blooming and Sunlight*

The writer performed the following experiment in order to find out the relation between the blooming of oat flowers and the sunlight. He made a cardboard cylinder 9 inches long and 3 inches diameter and 5 heads of oats which were to bloom on the day were taken and put into 4 glass bottles which were again put into the cylinder made of cardboard, the sunlight being thus intercepted. The cylinder was placed in the experimental room together with the remaining one as a control. The flowers of those heads were examined after a certain period of time; the results are shown in Table 21.

TABLE 21. *Number of blooming flowers when plant was exposed to sunlight and when it was intercepted*

Date (1929)	Time at which heads were examined	Number of blooming flowers				
		Control (one head)	Sunlight intercepted			
			No. 1	No. 2	No. 3	No. 4
	p.m.					
July 8	3.00	3	2	4	5	3
,, 9	3.30	4	3	5	4	4
,, 10	3.30	2	3	2	3	1
,, 11	4.00	3	2	3	4	2
,, 12	5.00	5	4	4	5	3
,, 13	4.00	3	1	4	2	4
,, 14	4.00	4	5	3	3	2

These results show that even when the sunlight was intercepted blooming took place. The writer also observed that when a head of oats growing on the experimental plot was put into a camera of small size, blooming took place in just the same manner as in those growing outside. But the blooming in this case occurred later than those outside on a clear and hot day, and the blooming occurred almost at the same time as those outside on a cloudy day. The writer considers that this is due to the changes of temperature.

While the experiment (II. B. 1.) was being performed to find the relation between temperature and blooming, it was observed that the oats which had been put into a thermostat under ground bloomed at night between 8 and 10 o'clock. And also in the dark room blooming took place as in the case of those growing outside when other conditions were suitable. These facts confirm that sunlight is not necessary for the blooming of oats.

7. *Summary*

1. The range of temperature on the days on which blooming occurred was 15–32°C. for the highest temperature and 14–29.8°C. for the temperature at blooming time. The most suitable highest temperature was 27–29°C. and the best temperature for blooming time 24–26°C.

2. There is an intimate relation between the changes of temperature in the day and blooming. Blooming began to take place when the difference between the temperature at about 8 a.m. and the highest temperature of the day was 3–8°C., and when temperature fell 1.0–2.5°C. within an hour or two after the time of the day's highest temperature.

3. There were days on which no blooming occurred even though the temperature was within the range of blooming, and the changes of temperature on these days were quite slight when compared with those on a blooming day.

4. There is a close relation between blooming time and the time of the highest temperature of the day. When the highest temperature was attained early, the blooming time was also early, and when the highest temperature came late, the blooming time was also late.

5. The range of minimum humidity on blooming days was 37.7–91.0% and 41.5–100% when the humidity at the time of blooming was considered. The most suitable minimum humidity was 50–60%, the best humidity at the time of blooming was 60–70%.

6. There is a close relation between blooming and changes of humidity. Blooming occurred when the difference between the morning humidity and the minimum humidity was 10–20%, and when humidity increased by 3–7% within an hour or two after the time of the minimum humidity.

7. There were days on which blooming did not take place although the humidity of the day was within the range suitable for blooming.

When the humidity on these days was compared with that of blooming days, there were generally found to be less changes in humidity on the non-blooming days.

8. There is an intimate relation between the blooming time and the time of the minimum humidity of the day. When the minimum humidity was reached early, blooming also took place early, and on the contrary, when the former came late, the latter also took place late.

9. There were many rainy days during the observation of blooming under natural conditions, and on some of these rainy days no blooming occurred at all and on others blooming occurred just as it did on the days when it did not rain. Therefore rainfall itself has no direct influence on blooming.

10. There is no direct relation between the force of wind and blooming and its time.

11. When there was more sunshine, more blooming occurred, but there were instances in which no blooming took place in spite of much sunshine. Therefore no direct relation exists between sunshine and blooming.

12. Blooming occurred as under natural conditions even when sunlight was intercepted and therefore there is no relation between sunlight and blooming.

B. Control of Blooming of Oat Flowers

The results of the observations stated in the foregoing chapter show that among the external factors the variations of temperature and humidity have an intimate relation to blooming. In order to prove this fact further, the writer performed the following described experiments regarding the control of the time of blooming during the day and control of blooming on blooming and non-blooming days by temperature and humidity and also to find out which of these two factors has a particularly important relation to blooming.

1. Blooming controlled by temperature

(i) Control of Blooming Time during the Day

The writer stated in his preliminary report[26] that by changing temperature he succeeded in controlling the time of blooming during the day and in controlling the blooming on blooming days and non-blooming

days. The writer repeated the same experiments to render those findings more certain. The results of these experiments are recorded below.

Materials and Methods

In 1927 and 1928, the writer picked those heads which were to bloom between 5 a.m. and 2 p.m. in the day, and put them in a small glass bottle containing water and changed the temperature by the following methods:—

1:—A head which was put in a small glass bottle was put into a thermostat having a temperature higher than that of atmosphere at the time when this head was cut. At a difinite hour it was again removed to a thermostat the temperature of which was low. It is, however, necessary here to describe two different ways of removing the materials from the atmospheric temperature to a higher one.

a:—To put into a thermostat raised suddenly to a high tempara-ture.

b:—To put into a thermostat the temperature of which is made gradually higher.

2:—The methods of changing the temperature were similar to those of 1 b, but the time at which the heads were cut and put into thermostat was earlier than in 1 b.

3:—Heads in natural condition were cut when the flowers had not yet opened. They were put into a small glass bottle and immediately removed into a thermostat the temperature of which was lower than it was outside at that time.

The results of the experiments

No. 1:—Table 22 which follows shows the results of the experiments conducted according to the method given in 1 a. In each case the blooming occurred at hours different from the natural blooming time of the day. Blooming took place sooner or later than the natural blooming time of that day according to the time when the temperature was altered from high degrees to low ones.

TABLE 22. *Results of the experiments on the control of blooming time conducted according to method No. 1 a*

Experiment 1

(July 16, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed									Number of heads used in the experiments	Time of natural blooming
		High	Low	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
8 a.m. 24.8°C.	10.00 a.m.	27°	22°	0	0	0	3	0	0	0	0	0	1	2.15 p.m.
		30°	22°	0	0	0	6	0	0	0	0	0	1	
	12.00 m.	27°	22°			2	0	0	0	0	0	0	1	
		30°	22°			0	0	0	0	0	0	0	1	
	2.00 p.m.	27°	22°					5	0	0	0	0	1	
		30°	22°					0	1	0	0	0	1	
	4.00 p.m.	27°	22°							2	0	0	1	
		30°	22°							1	0	0	1	
	6.00 p.m.	27°	22°									0	1	
		30°	22°									7	1	

TABLE 22. (*Continued*)
Experiment 2
(*July 2, 1928*)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	12.30 p.m.	1.30 p.m.	2.30 p.m.	3.30 p.m.	4.30 p.m.		
8.30 a.m. 22°C.	11.30 a.m.	26°	20°	5	4	0	0	0	2	2.15 p.m.
		24°	20°	8	4	0	0	0	2	
	1.30 p.m.	26°	20°			12	0	0	2	
		24°	20°			14	0	0	2	
	2.30 p.m.	26°	20°				4	0	2	
		24°	20°				6	0	2	

TABLE 22. (*Continued*)
Experiment 3
(July 17, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed								Number of heads used in the experiments	Time of natural blooming
		High	Low	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
9 a.m. 23.2°C.	11.00 a.m.	29°	23°	0	0	6	3	0	0	0	0	1	1.30 p.m.
		31°	23°	0	0	8	4	0	0	0	0	1	
	1.00 p.m.	29°	23°			7	1	0	0	0	0	1	
		31°	23°			0	7	0	0	0	0	1	
	3.00 p.m.	29°	23°					4	0	0	0	1	
		31°	23°					7	0	0	0	1	
	5.00 p.m.	29°	23°							3	0	1	
		31°	23°							3	0	1	

TABLE 22. (*Continued*)

Experiment 4

(July 5, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed				Number of heads used in the experiments	Time of natural blooming
		High	Low	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
9 a.m. 21°C.	3.00 p.m.	29°	19°	2	0	0	0	2	1.45 p.m.
	4.00 p.m.	29°	19°		2	1	0	2	

TABLE 22. (*Continued*)

Experiment 5

(July 6, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	2 p.m.	4 p.m.	4 p.m.	5 p.m.	6 p.m.		
9 a.m. 20°C.	1.00 p.m.	22.8°	19°	6	0	0	0	0	2	2.20 p.m.
	3.00 p.m.	22.8°	19°			2	0	0	2	
	4.00 p.m.	22.8°	19°					0	2	

TABLE 22. (*Continued*)
Experiment 6
(July 9, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed							Number of heads used in the experiments	Time of natural blooming
		High	Low	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
9 a.m. 21°C.	12.00 m.	29°	19°	10	10	0	0	0	0	0	5	2.20 p.m.
	2.00 p.m.	29°	19°				22	0	0	0	5	
	4.00 p.m.	29°	19°						31	0	5	
	5.00 p.m.	29°	19°							17	5	

TABLE 22. (*Continued*)
Experiment 7
(July 10, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed						Number of heads used in the experiments	Time of natural blooming
		High	Low	1.30 p.m.	2.30 p.m.	3.30 p.m.	4.30 p.m.	5.30 p.m.	5.30 p.m.		
9.30 a.m 25°C.	12.30 p.m.	32°	20°	0	2	0	0	0	0	1	1.15 p.m.
		28°	20°	4	0	0	0	0	0	1	
	1.30 p.m.	32°	20°		0	4	1	0	0	1	
		28°	20°		3	0	0	0	0	1	
	2.30 p.m.	32°	20°			0	3	0	0	1	
		28°	20°			6	0	0	0	1	
	3.30 p.m.	32°	20°				2	7	0	1	
		28°	20°				2	0	0	1	

TABLE 22. (*Continued*)
Experiment 8
(July 16, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed						Number of heads used in the experiments	Time of natural blooming
		High	Low	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
10 a.m. 27.2°C.	12.00 m.	32°	22°	0	1	0	0	0	0	1	2.30 p.m.
	2.00 p.m.	32°	22°			1	0	0	0	1	
	4.00 p.m.	32°	22°				1	0	0	1	
	6.00 p.m.	32°	22°						3	1	

TABLE 22. (*Continued*)
Experiment 9
(July 7, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
10 a.m. 21.4°C.	1.00 p.m.	30°	19°	9	0	0	0	0	3	2.45 p.m.
	2.00 p.m.	30°	19°		8	0	0	0	3	
	3.00 p.m.	30°	19°			12	0	0	3	

TABLE 22. (*Continued*)
Experiment 10
(July 17, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
11 a.m. 25.1°C.	1.00 p.m.	29°	23°	2	1	0	0	0	1	1.30 p.m.
		31°	23°	1	2	0	0	0	1	
	3.00 p.m.	29°	23°			4	0	0	1	
		31°	23°			4	0	0	1	
	5.00 p.m.	29°	23°					3	1	
		31°	23°					1	1	

TABLE 22. (*Continued*)
Experiment 11
(July 9, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	3 p.m.	4 p.m.	5 p.m.	6 p.m.	7 p.m.		
11 a.m. 23.8°C.	2.00 p.m.	29°	19°	1	12	0	0	0	5	2.20 p.m.
	4.00 p.m.	29°	19°			14	0	0	5	
	5.00 p.m.	29°	19°				18	0	5	

TABLE 22. (*Continued*)
Experiment 12
(July 16, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed			Number of heads used in the experiments	Time of natural blooming
		High	Low	5 p.m.	6 p.m.	7 p.m.		
12 m. 27.4°C.	4.00 p.m.	30°	22°	3	0	0	1	1.30 p.m.
	6.00 p.m.	30°	22°			2	1	

TABLE 22. (*Continued*)
Experiment 13
(July 4, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed					Number of heads used in the experiments	Time of natural blooming
		High	Low	4 p.m.	5 p.m.	6 p.m.	7 p.m.	10.30 p.m.		
12 m. 22.8°C.	3.00 p.m.	25°	19°	5	0	0	0	0	2	1.15 p.m.
	4.00 p.m.	25°	19°		4	0	0	0	2	
	9.00 p.m.	25°	19°					7	2	

TABLE 22. (*Continued*)
Experiment 14
(July 17, 1927)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed				Number of heads used in the experiments	Time of natural blooming
		High	Low	4 p.m.	5 p.m.	6 p.m.	10.30 a.m. (Jul. 18)		
1.00 p.m. 24.6°C.	3.00 p.m.	29° 31°	23° 23°	1 4	0 0	0 0		1 1	1.30 p.m.
	5.00 p.m.	29° 31°	23° 23°			2 4		1 1	
	9.30 a.m. (Jul. 18)	29° 31°	23° 23°				4 1	1 1	

TABLE 22. (*Concluded*).
Experiment 15
(July 9, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed				Number of heads used in the experiments	Time of natural blooming
		High	Low	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
1 p.m. 25.5°C.	2.00 p.m.	29°	19°	1	6	0	0	5	2.20 p.m.
	4.00 p.m.	29°	19°			9	0	5	
	5.00 p.m.	29°	19°				6	5	

No. 2:—Table 23 shows the results of the experiments conducted according to method 1. b. These experiments are different from 1. a. method in the changing of the temperature, but as in No. 1 the hours of blooming can be controlled in each case.

TABLE 23. *Results of the experiments on the control of blooming time conducted according to method No. 1 b*

Experiment 16

(July 18, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed								Number of heads used in the experiments	Time of natural blooming
		High	Low	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
8 a.m. 24°C.	10.00 a.m.	26°	20°	0	0	8	0	0	0	0	0	2	1.30 p.m.
	12.00 m.	26°	20°			8	0	0	0	0	0	2	
	2.00 p.m.	26°	20°					9	0	0	0	2	
	4.00 p.m.	26°	20°							7	0	2	
	5.00 p.m.	26°	20°								6	2	

TABLE 23 (*Continued*)
Experiment 17
(July 18, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)			Blooming time and number of flowers which bloomed						Number of heads used in the experiments	*Time of natural blooming*
		Low	High	Low	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
8 a.m. 24°C.	12.00 m.	26°	28°	22°	0	6	0	0	0	0	2	1.30 p.m.
	2.00 p.m.	26°	28°	22°			11	0	0	0	2	
	4.00 p.m.	26°	28°	22°					14	0	2	
	5.00 p.m.	26°	28°	22°						16	2	

TABLE 23 (*Continued*)
Experiment 18
(July 18, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	*Temperature of the thermostat* (C.)				Blooming time and number of flowers which bloomed				Number of heads used in the experiments	Time of natural blooming
						3 p.m.	4 p.m.	5 p.m.	6 p.m.		
8 a.m. 24°C.	2.00 p.m.	26°	28°	32°	26°	10	0	0	0	2	1.30 p.m.
	4.00 p.m.	26°	28°	32°	26°			3	5	2	
	5.00 p.m.	26°	28°	32°	26°				6	2	

TABLE 23 (*Continued*)

Experiment 19

(July 18, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed						Number of heads used in the experiments	Time of natural blooming
		High	Low	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.		
10 a.m. 24°C.	12.00 m.	28°	26°	4	9	0	0	0	0	2	4.30 p.m.
	2.00 p.m.	28°	26°			12	0	0	0	2	
	4.00 p.m.	28°	26°					10	0	2	
	5.00 p.m.	28°	26°						3	2	

TABLE 23 (*Continued*)

Experiment 20

(July 18, 1928)

Time and temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)			Blooming time and number of flowers which bloomed				Number of heads used in the experiments	Time of natural blooming
		Low	High	Low	2 p.m.	4 p.m.	5 p.m.	6 p.m.		
10 a.m. 24°C.	2.00 p.m.	28°	32°	26°	4	9	0	0	2	4.30 p.m.
	4.00 p.m.	28°	32°	26°			5	8	2	

No. 3:—The results given in Table 24 are obtained by method 2. These results show that when no change was made in temperature at all, blooming never occurred, but when there was change in temperature, every head bloomed before the time of the natural blooming. The earliest one began to open at 11.00 a.m. It took place about 4 hours earlier than the earliest under natural conditions.

TABLE 24. *Results of the experiments on the control of blooming time conducted according to method No. 3*

Experiment 21

(July 21, 1928)

Temperature outside at 5:30 a.m. ....16.3°C.

Blooming time	I		II		III		IV		V		VI	
	T[1]	B[2]	T	B	T	B	T	B	T	B	T	B
a.m.												
5.30	22°		24°		24°		22°		22°		19°	
7.00	22		24		24		22		22		19	
8.00	22		24		26		24		26		19	
9.00	22		24		26		26		28		19	
10.00	28		22		24		26		22		19	
10.30	27		22		20		26		22		19	
11.00	27		22		20		22		22		19	
11.30	26		22		20	1	22		22		19	
12.00	26		22	1	20	3	20		20		19	
p.m.												
0.30	25	5	22	1	20	2	20		20		19	
1.00	25	9	22	6	20	7	20		20		19	
1.30	24	3	22	13	20	15	20	7	20	8	19	
2.00	24	6	22	7	20	4	20	3	20	10	19	
2.30	22	4	22	2	20	1	20	2	20	3	19	
3.00	22		22	1	20		20	1	20	2	19	
3.30	22		22		20		20		20		19	
4.00	21		22		20		20		20		19	
Total		27		31		33		13		23		0
Number of heads used in the experiment	5		5		5		2		3		5	
Time of natural blooming	3 p.m.											

(1) T = Temperature of thermostat (c.)

(2) B = Number of flowers bloomed

TABLE 24. *(Concluded)*

Experiment 22

(July 24, 1928)

Temperature of outside at 5:00 a.m. ....20°C.

Blooming time	I		II		III		IV		V		VI		VII		VIII		IX		X	
	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B
a.m.	°		°		°		°		°		°		°		°		°		°	
5.00	20		20		24		24		24		24		22		22		20		15	
6.00	20		20		24		24		24		24		24		24		20		15	
7.00	20		20		24		24		27		27		27		27		20		15	
8.00	20		20		24		25		27		27		27		27		20		15	
9.00	31		31		20		15		24		15		20		15		20		15	
10.00	31		31		20		15		24		15		20		15		20		15	
11.00	21		15		20		15	**1**	24		15	**1**	20		15		20		15	
11.30	21	**2**	15		20		15	**1**	24		15	**4**	20		15	**2**	20		15	
12.00	21	**7**	15	**5**	20	**6**	15	**1**	24	**2**	15	**1**	20	**2**	15	**1**	20		15	
p.m.																				
0.30	21	**21**	15	**29**	20	**21**	15	**17**	24	**3**	15	**3**	20	**30**	15	**11**	20		15	
1.00	21	**9**	15	**2**	20	**4**	15	**6**	24	**5**	15	**17**	20	**5**	15	**6**	20		15	
1.30	21	**4**	15	**7**	20	**3**	15	**4**	24	**3**	15	**6**	20	**3**	15	**3**	20		15	
2.00	21		15	**1**	20	**1**	15	**1**	24	**1**	15		20	**1**	15		20		15	
2.30	21		15		20		15		24		15		20		15		20		15	
3.00	21		15		20		15		24		15		20		15		20		15	
3.30	21		15		20		15		24		15		20		15		20		15	
4.00	21		15		20		15		24		15		20		15		20		15	
Total		**43**		**44**		**53**		**31**		**22**		**32**		**41**		**23**		**0**		**0**
Number of heads used in the experiment	5		5		5		5		5		5		5		5		5		5	
Time of natural blooming	2.30 p.m.																			

No. 4:—Table 25 gives the result of the experiment conducted according to method 3 as described above. These results show that heads put into the lower temperature after 9.00 a.m. bloomed earlier than the time of blooming under natural conditions. But those treated in the same way between 6.00 a.m. and 9.00 a.m. never bloomed. This is because there were slight differences between the outside and the thermostat's temperature.

TABLE 25. *Results of the experiments on the control of blooming time conducted according to method No. 3*

Experiment 23

(July 11, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed										Number of heads used in the experiment	Time of natural blooming
		9 a.m.	9.30 p.m.	10 p.m.	10.30 a.m.	11 a.m.	12 m.	12.30 p.m.	1 p.m.	2 p.m.	3 p.m.		
8.00 a.m. 22.0°	21.0°	0	0	0	0	0	0	0	0	0	0	2	2 p.m.
8.30 a.m. 24.3°	21.0°		0	0	0	0	0	4	0	0	0	2	
9.00 a.m. 25.8°	21.0°			0	0	0	0	5	0	0	0	2	
9.30 a.m. 26.8°	21.0°				0	0	0	6	0	0	0	2	
10.00 a.m. 26.8°	21.0°					0	0	0	5	0	0	2	
11.00 a.m. 27.4°	21.0°						0	0	4	0	0	2	
12.00 m. 28.1°	21.0°							0	0	3	0	2	
1.00 p.m. 28.5°	21.0°									5	0	2	
2.00 p.m. 25.[illegible]	21.0°										11	2	

TABLE 25—(*Continued*)

Experiment 24

(July 14, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed										Number of heads used in the experiment	Time of natural blooming
		7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.		
6.30 a.m. 18.8°	19.0°	0	0	0	0	0	0	0	0	0	0	2	4.15 p.m.
7.00 a.m. 20.5°	19.0°		0	0	0	0	0	0	0	0	0	2	
9.00 a.m. 23.1°	19.0°				0	0	0	1	4	0	0	2	
10.00 a.m. 24.1°	19.0°					0	0	3	5	0	0	2	
11.00 a.m. 25.5°	19.0°						0	5	5	0	0	2	
12.00 m. 26.2°	19.0°							0	8	0	0	2	
1.00 p.m. 26.3°	19.0°								7	0	0	2	
2.00 p.m. 26.4°	19.0°									15	0	2	
3.00 p.m. 27.0°	19.0°										15	2	

TABLE 25—(*Continued*)

Experiment 25

(July 13, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed							Number of heads used in the experiment	Time of natural blooming
		10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.		
9.00 a.m. 21.0°	21.0°	0	0	0	0	0	0	0	2	4.00 p.m.
10.00 a.m. 24.3°	21.0°		0	0	0	6	0	0	2	
11.00 a.m. 26.7°	21.0°			0	0	4	0	0	2	
12.00 m. 28.0°	21.0°				0	7	0	0	2	
1.00 p.m. 27.3°	21.0°					0	4	0	2	
2.00 p.m. 28.0°	21.0°						7	0	2	
3.00 p.m. 27.5°	21.0°							8	2	

TABLE 25—(*Continued*)

Experiment 26

(July 6, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed							Number of heads used in the experiment	Time of natural blooming
		11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.		
10.00 a.m. 21.1°	13.0°	0	0	3	0	0	0	0	3	2.45 p.m.
11.00 a.m. 21.8°	13.0°		0	3	0	0	0	0	3	
12.00 m. 21.8°	13.0°			7	0	0	0	0	3	
1.00 p.m. 21.8°	13.0°				5	0	0	0	3	
2.00 p.m. 21.7°	13.0°					9	0	0	3	

TABLE 25—(*Continued*)

Experiment 27

(July 10, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed				Number of heads used in the experiment	Time of natural blooming
		10.30 a.m.	11.30 a.m.	12.30 p.m.	1.30 p.m.		
9.30 a.m. 25.0°	21.0°	0	0	4	0	3	1.50 p.m.
10.30 a.m. 26.8°	21.0°		0	8	0	3	
11.00 a.m. 27.5°	21.0°		0	3	0	3	
11.30 a.m. 27.5°	21.0°			0	6	3	

TABLE 25—(*Continued*)

Experiment 28

(July 7, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed							Number of heads used in the experiment	Time of natural blooming
		10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.		
9.00 a.m. 19.5°	19.5°	0	0	0	0	0	0	0	3	2.45 p.m.
10.00 a.m. 20.0°	19.5°		0	0	0	0	0	0	3	
11.00 a.m. 22.4°	19.5°			0	4	3	0	0	3	
12.00 m. 22.5°	19.5°				6	1	0	0	3	
1.00 p.m. 23.2°	19.5°					7	0	0	3	
2.00 p.m. 23.2°	19.5°						13	0	3	

TABLE 25—(*Concluded*)

Experiment 29

(July 9, 1928)

Time and outside temperature when heads were put into the thermostat of low temperature (C.)	Temperature of the thermostat (C.)	Blooming time and number of flowers which bloomed							Number of heads used in the experiment	Time of natural blooming
		10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	5 p.m.		
9.00 a.m. 21.0°	20.0°	0	0	0	0	0	0	0	2	2.20 p.m.
10.00 a.m. 23.4°	20.0°		0	0	19	0	0	0	2	
11.00 a.m. 23.8°	20.0°			0	13	0	0	0	2	
12.00 m. 23.5°	20.0°				5	2	0	0	2	
1.00 p.m. 25.5°	20.0°					20	0	0	2	
2.00 p.m. 25.5°	20.0°						24	0	2	

(ii) Control of blooming on blooming and non-blooming days

According to the writer's observation, there are a number of days when no blooming takes place even though the day's temperature is in the range of blooming temperature on the blooming days, and on these days the variations of temperature are not so remarkable as compared with the days on which blooming occurs. Therefore the writer conducted experiments concerning the control of blooming, anticipating that, by adjusting the temperature, he could make flowers open on the days when no blooming would be supposed to occur and could suppress blooming on the days favourable for blooming. The results of the experiments, as described below, coincide with those already stated in his preliminary report.[20]

a :—Control of blooming on the days when blooming does not occur.

Materials and Method

Fully developed heads were cut on a day when no blooming was supposed to occur and put in a glass bottle. These were put for a certain length of time first into a thermostat the temperature of which was higher than the outside temperature at the time and then removed into a thermostat whose temperature was lower.

Results of the experiments

The results are shown in Table 26. Each experiment shows that, by adjusting temperature artificially, blooming can be made to occur on the days when flowers do not open under natural conditions. It is necessary to pay special attention to the fact that on these days high temperature does not merely cause flowering, but that only when temperature falls does blooming occur.

TABLE 26. *Results of experiments on the control of blooming on non-blooming days.*

Experiment 30

(July 8, 1928)

Time and outside temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed			Number of heads used in the experiment
		High	Low	1.00 p.m.	2.00 p.m.	3.00 p.m.	
10.00 a.m. 18.8°C.	12.00 p.m.	29°	20°	0	8	0	2
		26°	20°	0	6	0	2
	1.00 p.m.	29°	20°		12	0	2
		26°	20°		9	0	2
	2.00 p.m.	29°	20°			9	2
		26°	20°			8	2

TABLE 26—(*Continued*)

Experiment 31

(August 31, 1928)

Time and outside temperature when heads were put into the thermostat of high temperature	Time at which heads were transferred from the thermostat of high temperature to that of low	Temperature of the thermostat (C.)		Blooming time and number of flowers which bloomed			Number of heads used in the experiment
		High	Low	2.00 p.m.	3.00 p.m.	4.00 p.m.	
9.30 a.m. 23.8°C.	1.00 p.m.	28°	20°	4	2	0	5
•	1.00 p.m.	29°	20°	2	4	0	5

TABLE 26—(*Continued*)

Experiment 32

(September 1, 1928)

	Blooming time and number of flowers which bloomed								Number of heads used in the experiment
	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	Total	
A	0	0	0	3	0	0	0	3	2
B	0	0	0	2	0	0	0	2	2
C	0	0	0	0	0	0	0	0	2
D	0	0	0	0	0	0	0	0	2

A and B were put into a thermostat set at 25.5°C. at 10 a.m. when the outside temperature was 20°C., and then A was put in another thermostat set at 20°C. at 1 p.m. B was exposed to the high temperature of 29°C. from 12 m. to 1 p.m. and then to the lower temperature of 20°C., and C was kept in a thermostat set at 20°C. (the same temperature as outside) and no change in the temperature was made. D, which had been cut, was left on the plot in the natural temperature.

TABLE 26—(*Continued*)

Experiment 33

(September 12, 1928)

	Blooming time and number of flowers which bloomed									Number of heads used in the experiment
	10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	Total	
A				0	0	3	0	0	3	2
B	0	0	0	0	0	0	0	0	0	2
C	0	0	0	0	0	0	0	0	0	2

The heads A, B and C were cut at 9.30 a.m. when the temperature was 19.5°C. A was put in the thermostat set at 27°C. and at 12.30 p.m. it was lowered to 19°C. B was kept in the thermostat set at 19°C. all day, and C was left on the plot and exposed to the natural temperature.

TABLE 26—(*Continued*)

Experiment 34

(September 19, 1928)

Time		9.30 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	Total	Number of heads used in the experiment
I.	T(1)	25°	25°	25°	21°	21°	21°	21°	21°	21°		
	B(2)						6	0	0	0	6	3
II.	T	25°	25°	25°	25°	25°	21°	21°	21°	21°		
	B							5	0	0	5	3
III.	T	25°	25°	25°	25°	29°	21°	21°	21°	21°		
	B							7	0	0	7	3
IV.	T'(3)	17°	17.2°	16.4°	16°	16°	15.5°	15.4°	15.2°	15.2°		
	B										0	3

(1)——T Temperature of the thermostat (C.)
(2)——B Number of flowers opened
(3)——T' Natural temperature of the day

TABLE 26—(*Concluded*)

Experiment 35

(*September 21, 1928*)

Time		9 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	2.30 p.m.	3.30 p.m.	3.30 p.m.	4 p.m.	Total	Number of heads used in the experiment
I.	T(1)	19°	19°	19°	19°	28°	19°	19°	19°	19°		
	B(2)							0	2	0	2	2
II.	T	19°	19°	19°	19°	28°	28°	28°	19°	19°		
	B									3	3	2
III.	T'(3)	16.8°	18.5°	19°	19.5°	20.5°	20.5°	9.5°	19.8°	19.5°		
	B										0	2

(1)——T Temperature of the thermostat (C.)
(2)——B Number of flowers opened
(3)——T' Natural temperature of the day

b:—Control of blooming on the days when blooming occurs.

Heads were cut on a day when the external conditions were most favourable for normal blooming and were put into a thermostat whose temperature was almost the same as that outside at the time of cutting. In this case no flowers open, as is shown in the foregoing tables (Table 24. Ex. 21, Ex. 22. Table 25. Ex. 23, 24, 25, 28 and 29). Thus on the days for blooming, it can be checked by controlling the temperature.

2. *Blooming controlled by Humidity*

As is shown in the results of the observations described in the previous chapter, an intimate relation between the variations of humidity and blooming can be recognized, but the variations of humidity are caused only subordinately to those of temperature and it can be considered that they are not connected directly with blooming. The writer in order to make this point clear, conducted experiments concerning the blooming time and blooming in humid variations.

Materials and Methods

In this investigation, fully developed heads were used. Humidity is divided into the following three different kinds.

A:—Natural state of humidity inside a tent pitched in the experimental farm, in a dark room or in a thermostat. This is called "A" in the following.

B:—A small quantity of water is poured into a glass jar with a lid. In a little while the air in the jar is saturated with moisture. This state of humidity is hereafter called "B".

C:—A small quantity of calcium chloride (for drying) is poured into a glass jar and the air in it is made dry. This state of dryness is called "C".

The heads were cut at three different hours between 6 a.m. and 7 a.m., between 10 a.m. and 11 a.m. and between 1 p.m. and 2 p.m. and were put in small glass bottles. These bottles were put again into glass jars whose humidity was "B" and "C" respectively and were placed in the tent on the farm, in the dark room of the laboratory, and in the thermostat. At the same time the heads put into other small glass bottles were placed side by side with the glass jar in each of the three above mentioned places. The heads thus treated were subjected to the following variations in temperature and humidity.

From 6 a.m. to 7 a.m. the temperature is low and humidity high. When the heads cut during this time are put in small glass bottles which are put again into the glass jar of humidity "B" and are placed in the tent, the humidity increases a little, while in those put into the glass jar of humidity "C" it decreases. The temperature depends on that of the day as under natural conditions. Those put into the dark room are subject to the same changes in humidity as in the above case. The tem-

perature of the dark room is low and no changes are caused during the whole day. Those put into the thermostat are subjected to the same changes of humidity as in the case of the tent, but the temperature could be changed at will.

From 10 a.m. to 11 a.m. temperature rises a little and humidity decreases somewhat. The heads cut during this time and put into the glass jar of humidity "B" and then placed in the tent are subjected to the change of a little more increase in humidity and those heads in the glass jar of humidity "C" are affected by the changes caused by a decrease of humidity. The changes of temperature depend on those in the natural state of that day. Those placed in the dark room undergo the same changes in humidity as those put in the tent. The temperature in the dark room is lower than that from 10 a.m. to 11 a.m. and therefore the heads are affected by changes caused by its decrease. Those put into the thermostat are subject to the same effect as in the case of the tent. Its temperature can be adjusted at will.

From 1 p.m. to 2 p.m. temperature becomes much higher and humidity decreases very much. The heads put into the glass jar of humidity "B" in the tent undergo changes caused by a great increase of humidity and those in the glass jar "C" are affected by the changes caused by decrease. The temperature depends on the changes in natural state. Those heads placed in the dark room are affected by the same changes in humidity as caused in the tent; the temperature in the room is lower than outside at this time, therefore they are subjected to much greater changes caused by decrease than in the case from 10 a.m. to 11 a.m. Those put in the thermostat receive the same change of humidity as caused in the tent and temperature can be kept at the same degree as that outside and can be made higher than that.

The resnlts of the experiments

The results of the experiments conducted in the above described manner, are shown in Table 27.

The flowering time should be considered with reference to the results shown in Table 27. The natural flowering time on July 16th (Experiment 1) was 3:30 p.m. and the heads put in the tent on the farm bloomed almost at the same time as those in the natural coudition. Those put in the dark room began to bloom earlier than those in the natural condition.

TABLE 27. *Relation between blooming and humidity*

Experiment 1

(July 16, 1929)

The places in which heads were put	In shed on the experimental plot									In dark room									In thermostat						
Time at which heads were cut	6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			Control
Time \ Humidity	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed: p.m. 0.30										2	2	3													
1.00										1	6	2													
1.30										0	0	3	1	0	0										
2.00													8	8	8										
2.30													1	0	0										
3.00	5	0	0	2	0	0	0	0	0	0	0	0				10	17	10							
3.30	6	11	12	7	10	16	7	7	0							0	0	0							8
4.00	0	3	6	0	5	0	0	5	6																2
Total	11	14	18	9	15	16	7	12	6	3	8	8	10	8	8	10	17	10							10

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

TABLE 27—(*Continued*)

Experiment 2

(July 18, 1929)

	The places in which heads were put	In shed on the experimental plot									In dark room									In thermostat						
	Time at which heads were cut	6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m.			6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m.			6.00-7.00 a.m.			10.00-11.00 a.m.			Control
	Humidity / Time	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed	p.m. 1.00										0	2	4	3	7	2										
	1.30										3	3	0	8	9	8										
	2.00																20	15	14							
	2.30																1	15	1							
	3.00	6	0	0	9	0	0	7	0	0																
	3.30	6	15	15	1	22	15	0	9	5																17
	4.00	0	0	0	0	0	1	0	0	0																
	Total	12	15	15	10	22	16	7	9	5	3	5	4	11	16	10	21	30	15							17

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

TABLE 27—(*Continued*)

Experiment 3

(July 23, 1929)

The places in which heads were put		In shed on the experimental plot									In dark room									In thermostat						
Time at which heads were cut		6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m			6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m.			6.00-7.00 a.m.			10.00-11.00 a.m.			Cintrol
Humidity / Time		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed	p.m.																									
	1.00										0	0	0	16	7	16										
	1.30										0	0	0	0	0	0										
	2.00										0	0	0	0	0	0										16
	2.30										0	0	0	0	0	0										
	3.00										0	0	0	0	0	0	28	28	36							
	3.30										0	0	0	0	0	0	0	0	0							
	4.00										0	0	0	0	0	0	0	0	0							
	4.30										0	0	0	0	0	0	0	0	0							
	5.00										0	0	0	0	0	0	0	0	0	8	12	15				
	5.30										0	0	0	0	0	0	0	0	0	0	0	0				
	Total										0	0	0	16	7	16	28	28	36	8	12	15				16

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

TABLE 27—(*Concluded*)

Experiment 4

(July 25, 1929)

The places in which heads were put	In shed on the experimental plot									In dark room									In thermostat						
Time at which heads were cut	6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			Control
Time \ Humidity	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed: p.m. 1.00													0	0	0										
1.30										0	0	0	5	1	0										
2.00										0	0	0	9	3	3										
2.30										0	0	0	8	10	14										
3.00										0	0	0	0	0	0										
3.30	9	0	0	9	0	0																			16
4.00	5	14	8	8	13	19																			4
4.30	1	7	4	0	0	0													9	10	8	11	0	0	
Total	15	21	12	17	13	19				0	0	0	22	14	17				9	10	8	11	0	0	20

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

This fact could be proved true on the other days (July 18th and 25th (Experiments 2 and 4.)). When the heads which were cut at different times and put into jars of different humidity and then placed in the tent, were observed, there could be perceived no difference between those cut early in the morning and those cut in the afternoon. In the case of dark room, the earlier they were put into it, the sooner they bloomed. In the natural condition they bloomed at 3:30 p.m. on July 16, but those put into the low temperature from between 6 and 7 a.m. bloomed at 12:30 p.m. and those between 1 p.m. and 2 p.m. bloomed at 3 p.m. This shows that they all bloomed earlier than the natural flowering time.

Next, it was investigated whether blooming occurs or not as a result of changes in humidity, and the result was that, whenever the heads in natural condition bloomed, those placed in the tent on the farm did also, regardless of the time when changes in humidity took place and of the differences in humidity. But it was not so with those placed in the dark room. Difference arises according to the time when changes in humidity take place. When changes in humidity took place between 10 and 11 a.m. and between 1 and 2 p.m. every head bloomed, but when changes took place between 6 and 7 a.m. the blooming varied according to days. As shown in Table 27, on July 16 and 18 blooming occurred in the three different humidities but on July 23 and 25 no blooming took place in them.

From these points it can be understood that blooming does not depend on humidity, but on something else. On the other hand, from the view point of temperature, on the days (July 23rd and 25th in Table 27) when no blooming occurred in the dark room (heads were cut early in the morning and put in bottles B and C) the temperature at 6 a.m. on July 23rd was 20.8°C and at 7 a.m. on July 25th was 22°C. These were almost the same as the temperature in the dark room which was 21°C. On the days (July 16, 18), when blooming occurred the temperature of the early morning was 26°C. which was much higher than that of the dark room, temperature of which being 23°C. This fact shows that on the days when no blooming occurs, there is little variation in temperature and that in the latter case the variation in temperature is great. Therefore it can be considered that blooming is caused not by the variation of humidity, but by that of temperature.

3. Summary

1. Experiments were conducted on the blooming of oats and the control of the time of blooming by the variation in temperature and humidity.

2. Blooming and the time of blooming can be controlled by changing temperature, but can not be controlled by changing humidity.

3. When the heads in the blooming stage are cut at different times and are first put in the higher temperature and then in the lower one, they can be made to bloom 3 or 4 hours earlier or later than the natural blooming time.

4. When the heads are put in a lower temperature than that at the time they are cut, they can be made to bloom 2 hours earlier than the natural blooming time.

5. By adjusting temperature oats can be made to bloom on days when no blooming occurs under the natural conditions.

6. By adjusting temperature the blooming can be checked.

7. The heads in the blooming stage are cut and put in the natural state of humidity in the air, in a glass jar which is saturated with moisture and in a glass jar which is made dry with calcium chloride. While there is no difference with regard to the time of blooming under those states of humidity, when the time of blooming arrives the heads in the 3 different humidities all bloom and if no blooming occurs it is the same in all three humidities.

III. Physiological studies on the Blooming of Oat Flowers

The writer found as the results of his observations and experiments given in chapter II, that variations in temperature have an intimate relation with blooming, and from this fact it follows that there must also be a very close relation between the swelling of the lodicules which is the direct cause of blooming and variations in temperature. In order to determine whether the variations of temperature are the direct cause of the swelling of the lodicules or not, the writer studied on the relation between the opening and closing of stomata and also the relation between blooming and absorption of water by the plant. Further, the writer studied on the relation between the morphological and chemical changes of the lodicules at the time of blooming and the temperature in order

to make clear the relation between temperature and physiology of blooming. The following are the results of these studies:—

1. Relation between the Opening and Closing of the Stomata and Temperature, Humidity and Blooming

Materials and Method

Oats in blooming stage were used as materials and the opening and closing of stomata on the dorsal side of the uppermost leaf of the main stem were carefully observed. The method of observation was by the use of cobaltchloride paper. This method was applied three times on the same leaf and its value was determined by their average. The writer observed it every hour between 9 a.m. and 4:30 p.m. The degrees of the opening and closing were shown by the length of time required for the deep standard colour to change to light standard colour.

Results of the experiment

From the results set down in Table 28, it is evident that stomata become wider as temperature rises and humidity decreases and they become smaller after the highest temperature with the least humidity of the day. That is to say, the opening and closing of stomata have parallel relation with temperature and humidity. Regarding such parallel relation between the environmental factors and transpiration BRIGGS and SCHANTZ[3] performed experiments with oats and they too obtained the same result as the writer did. As blooming occurs while the temperature of the day is declining from its highest point so the same thing happens while the stomata are closing. That is to say, when the quantity of water transpired from the stomata decreases, blooming occurs.

Therefore, it seems as if there exists a relation between blooming and the opening and closing of stomata, but the results of the experiment "on the relation between blooming and absorption" as shown in the following section proves that there is no direct relation between them.

2. Relation between Blooming and Absorption of Water by Plants

The results of the experiment reported in the previous section have shown that when temperature rises and stomata open wider and the transpiration from plants increases more and more, plants absorb more

TABLE 28. *Relation between the opening and closing of stomata and temperature, humidity and blooming 1928*

Date	Time	Length of time in seconds required for deep standard colour to change to lightstandard colours: I	II	III	Average	Temperature (C.)	Humidity (%)	Time of blooming
September 4.	9.30 a.m.	48 9/19	45 17/20	47 4/5	47 31/60	26.5°	75	
	10.30 a.m.	43 9/10	46 9/10	41 3/5	44 2/15	27.6	66	
	11.30 a.m.	51 7/10	47 9/10	69 3/10	56 9/10	27.7	71	3.00 p.m.
	1.30 p.m.	77 3/5	73 9/10	75	75 1/2	28.2	69.5	
	2.30 p.m.	78 2/5	76 1/4	77 1/10	77 1/4	26.9	74	
	3.00 p.m.	80 2/5	84 3/5	88 3/10	84 1/10	26.0	80	
September 5.	10.00 a.m.	61	64	62 7/10	62 17/30	27.2	68	
	11.00 a.m.	52 1/10	52 1/10	53 1/5	52 7/15	27.4	65	
	12.00 a.m.	46 7/10	41 1/4	45 9/10	45 11/60	28.0	65	
	1.00 p.m.	54 3/4	60 4/5	63 7/10	60 11/12	27.6	67.5	3.00 p.m.
	2.00 p.m.	67 1/5	67 7/20	69 1/10	68 19/60	27.0	70	
	3.00 p.m.	80 1/2	80 3/4	77 3/20	79 11/15	25.5	80	
	4.00 p.m.	96 1/5	82 1/5	90 1/5	89 59/60	24.5	86	
September 6.	10.00 a.m.	70 7/10	70 1/4	71 7/10	70 53/60	27.0	74	
	11.00 a.m.	61 3/20	58 1/4	64 9/10	61 13/30	26.7	76	
	12.00 a.m.	63 7/10	62 1/2	69 3/5	65 4/15	25.9	78	2.00 p.m.
	1.00 p.m.	62 2/5	67 4/5	71 4/5	68 8/15	26.0	77.5	
	2.00 p.m.	75	81 7/10	70 9/10	75 9/10	26.0	78	
September 7.	10.00 am.	67 9/10	71 1/10	67	68 1/3	27.5	67	
	11.00 a.m.	60 1/10	71 7/10	58	63 4/15	28.6	60	
	12.00 a.m.	66 1/2	70 1/10	70 2/5	68 2/3	29.0	54	3.00 p.m.
	1.00 p.m.	70 1/2	69 1/10	69 2/5	69 1/3	30.0	48	
	2.00 p.m.	66 7/10	65 1/4	69 3/5	66 11/60	30.4	51.5	
	3.00 p.m.	74 3/5	76 9/10	75 3/10	75 3/5	28.3	62	
September 8.	10.00 a.m.	102 2/5	92 3/5	100 1/10	98 11/30	19.0	52	
	12.00 a.m.	101 1/10	101 3/5	102 2/5	101 7/10	19.0	59	
	1.00 p.m.	125 7/10	125 2/5	125 3/10	125 7/15	20.2	58	3.00 p.m.
	2.30 p.m.	163 1/10	155 9/10	161 3/5	160 1/5	19.9	54	
	3.30 p.m.	198 1/4	191 2/5	178 9/10	189 31/60	19.0	59	
	4.00 p.m.	247 2/5	277	271 2/5	265 4/15	18.2	67	
Septemfler 22.	9.30 a.m.	166 2/5	165 7/10	169 2/5	167 1/6	18.3	59	
	1.30 a.m.	112	110 9/10	110 7/10	111 1/5	18.8	55.5	
	12.30 p.m.	106 9/10	108 4/5	108 17/20	106 51/60	19.6	58	3.00 p.m.
	2.00 p.m.	158 2/5	154 1/4	158 3/10	156 59/60	18.5	62	
	3.00 p.m.	137 3/5	131 4/5	111	126 11/15	19.0	63	
	4.00 p.m.	145	144 2/5	147 3/4	145 43/60	17.8	70	

water from the earth. But from this fact alone it can not be asserted that the increase of water has a direct relation with blooming, because the writer often observed in his experiment that the heads which were put into the bottle containing no water bloomed like those in the natural condition. From this fact, it seems that oats do not require much water at the time of blooming. The writer, therefore in order to make this relation clear, conducted the following experiments on the relation between absorption of water and blooming.

Materials and Method

Three heads of oats were put into each of the bottles, one of which contained some water. These were again put into glass jars, one of which had been saturated with moisture and the other was dry. These were ararnged under different environmental conditions, temperature was controlled and blooming was observed. (See Chapter II)

The results of experiment

The results are shown in Table 29. When the blooming of a head put into the bottle containing some water and that of another head put into the bottle containing no water are compared, under favourable environmental conditions the former never fails to bloom, but the latter sometimes blooms and sometimes does not. When heads are cut and put into the bottles and then exposed to the atmosphere, those in the bottle with water bloom, but those in the bottle with no water do not bloom. But those cut when the hours of blooming of the day approach, bloom when treated in the latter way. However, those put into the glass jars bloomed alike no matter whether water was present or not. When the heads are exposed to the atmosphere those in the bottle containing no water do not bloom because the transpiration from the head is so great that the head withers from the lack of water supplied from below. On the other hand, those in the bottles containing water do not wither, but bloom as usual because water is supplied from below. Inside the glass jars which are saturated with moisture, the humidity is high and there is no air current and so no water is lost from the plant, and those in the bottles containing no water need not be supplied water from the bottom, for blooming without withering away.

In the glass jars whose air is dried, those kept in the small bottle with no water in it, though there is no water to make up for the loss, do not wither. This is because, owing to the extraordinary changes in

TABLE 29. *Comparison of the blooming in the bottles which contain some water with blooming in others which contain no water under different humidities in shed and in room of low temperature*

Experiment 1 (July 16, 1929)

Places in which heads were put		In shed on the experimental plot									In room of low temperature									In thermostat						Control
Time at which heads were cut		6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			
Time (Number of flowers bloomed)	Humidity	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
p.m. 0.30	I										2	2	3													
	II										0	2	0													
1.00	I										1	6	2													
	II										0	6	0													
1.30	I										0	0	3	1	0	0										
	II										0	0	6	2	0	0										
2.00	I													8	8	8										
	II													7	10	9										
2.30	I													1	0	0										
	II													2	0	0										
3.00	I	5	0	0	2	0	0	0	0	0	0	0	0				10	17	10							
	II	0	0	0	0	0	0	0	0	0							13	10	10							
3.30	I	6	11	12	7	10	16	7	7	0							0	0	0							8
	II	0	7	3	0	12	3	5	4	0							1	0	0							
4.00	I	0	3	6	0	5	0	0	5	6																2
	II	0	4	7	0	5	0	9	2	11																
Total	I	11	14	18	9	15	16	7	12	6	3	8	8	10	8	8	10	17	10							10
	II	0	11	10	0	17	3	14	6	11	0	9	6	11	10	9	14	10	10							

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

I = The bottle which contained some water.
II = The bottle which contained no water.

TABLE 29—(*Continued*)

Experiment 2 (*July 18, 1929*)

Places in which heads were put		In shed on the experimental plot									In room of low temperature									In thermostat						
Time at which heads were cut		6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m.			6.00-7.00 a.m.			10.00-11.00 a.m.			1.00-2.00 p.m.			6.00-7.00 a.m.			10.00-11.00 a.m.			Control
Time \ Humidity		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed: p.m. 1.00	I										0	2	4	3	7	2										
	II										0	1	1	0	0	1										
1.30	I										3	3	0	8	9	8										
	II										0	3	1	0	4	1										
2.00	I																20	15	14							
	II																10	10	8							
2.30	I																1	15	1							
	II																10	10	0							
3.00	I	6	0	0	9	0	0	7	0	0																
	II	0	0	0	0	0	0	3	1	2																
3.30	I	6	15	15	1	22	15	0	9	5																17
	II	0	9	0	0	16	10	0	10	5																
4.00	I	0	0	0	0	0	1	0	0	0																
	II	0	1	2	0	1	1	0	0	0																
Total	I	12	15	15	10	22	16	7	9	5	3	5	4	11	16	10	21	30	15							17
	II	0	10	2	0	17	11	3	11	7	0	4	2	0	4	2	20	20	8							

A = Natural state *of humidity.*
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

I = The bottle which contained some water.
II = The bottle which contained no water.

TABLE 29—(*Continued*)

Experiment 3 (July 23, 1929)

	Places in which heads were put		In shed on the experimental plot									In room of low temperature									In thermostat						
	Time at which heads were cut		6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			Control
	Time	Humidity	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Number of flowers bloomed	p.m. 1.00	I										0	0	0	16	7	16										
		II										0	0	0	14	14	14										
	1.30	I										0	0	0	0	0	0										
		II										0	0	0	0	0	0										
	2.00	I										0	0	0	0	0	0										16
		II										0	0	0	0	0	0										
	2.30	I										0	0	0	0	0	0										
		II										0	0	0	0	0	0										
	3.00	I										0	0	0	0	0	0	28	28	36							
		II										0	0	0	0	0	0	42	31	27							
	3.30	I										0	0	0	0	0	0	0	0	0							
		II										0	0	0	0	0	0	0	0	0							
	4.00	I										0	0	0	0	0	0	0	0	0							
		II										0	0	0	0	0	0	0	0	0							
	4.30	I										0	0	0	0	0	0	0	0	0							
		II										0	0	0	0	0	0	0	0	0							
	5.00	I										0	0	0	0	0	0	0	0	0	8	12	15				
		II										0	0	0	0	0	0	0	0	0	15	3	6				
	5.30	I										0	0	0	0	0	0	0	0	0	0	0	0				
		II										0	0	0	0	0	0	0	0	0	0	0	0				
	Total	I										0	0	0	16	7	16	28	28	36	8	12	15				16
		II										0	0	0	14	14	14	42	31	27	15	3	6				

A = **Natural state of humidity.**
B = **The air in the bottle is saturated with moisture.**
C = **The air in the bottle is made dry.**

I = The bottle which contained some water.
II = The bottle which contained no water.

TABLE 29—(*Concluded*)

Experiment 4 (July 25, 1929)

Places in which heads were put		In shed on the experimental plot									In room of low temperature									In thermostat						Control
Time at which heads were cut		6.00–7.00 a.m.			10.00–11.00 a.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 p.m.			1.00–2.00 p.m.			6.00–7.00 a.m.			10.00–11.00 a.m.			
Time (Number of flowers bloomed)	Humidity	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
p.m. 1.00	I													0	0	0										
	II													0	0	0										
1.30	I										0	0	0	5	1	0										
	II										0	0	0	0	1	0										
2.00	I										0	0	0	9	3	3										
	II										0	0	0	1	3	9										
2.30	I										0	0	0	8	10	14										
	II										0	0	0	5	4	4										
3.00	I										0	0	0	0	0	0										
	II										0	0	0	10	0	0										
3.30	I	9	0	0	9	0	0																			16
	II	0	0	0	0	0	0																			
4.00	I	5	14	8	8	13	19																			4
	II	0	14	5	10	10	15																			
4.30	I	1	7	4	0	0	0													9	10	8	11	0	0	
	II	0	6	12	0	5	1													19	11	14	2	0	0	
Total	I	15	21	12	17	13	19				0	0	0	22	14	17				9	10	8	11	0	0	20
	II	0	20	17	10	15	16				0	0	0	16	8	13				19	11	14	2	0	0	

A = Natural state of humidity.
B = The air in the bottle is saturated with moisture.
C = The air in the bottle is made dry.

I = The bottle which contained some water.
II = The bottle which contained no water.

humidity, the stomata close, the transpiration decreases and there is no current of air, and therefore the loss of water from the plant body is very slight.

From the above mentioned experimental results, the writer has come to the following conclusion regarding the relation between blooming and absorption of water: a great quantity of water is not necessary to be taken up from the earth for the swelling of lodicules when blooming occurs, but the plant needs only enough water to keep itself from withering away.

3. *Changes in the Forms of the Lodicules at the Time of Blooming of Oats and their Relation to Temperature*

The writer recognized through the experiments described in sections 1 and 2 that the variation of temperature is the direct cause of blooming. Therefore, in order to make clear the relation between the swelling of lodicules, which is thought to be the mechanic action of blooming and variation of temperature, observation was made upon the morphological changes of lodicules at the time of blooming.

Materials and Methods

It may suffice to observe and compare the form of the lodicules at the three periods; before, during and after blooming. As the nature of this experiment makes it impossible to observe these three forms by using the same lodicules, so for those before blooming, according to the results of the previous observations on the order of blooming (Sec. 2. Chap. 1.), the lodicules in the order of blooming on the day were chosen and put into use.

The results

(i) Forms of lodicules of oats before blooming

The lodicules of oats 4–5 hours prior to blooming have forms as represented by Figs. 3 and 4 in Plate I. The form of lodicules seen from the front is shown in 3.A. and from the back in 4.A. The two lodicules are joined at their base. When these are separated, the forms appear as shown in Figs. 3.B. and 4.B. The form is flat and slender and the size is 2.11 mm. in length, 0.80 mm. in width and 0.50 mm. in thickness. The contents can be seen by the naked eye. The upper part is colourless and transparent while the lower part is of yellowish brown colour. There is a "hinter zahn" on the edge at the back which is

flat in form and of the same nature as the upper half of the lodicules as found by HACKEL[7] in the lodicule of *Festuca gigantea*.

(ii) Forms of lodicules of oats at the time of blooming

The forms of lodicules at the time of blooming are shown in Figs. 5 and 6 in Plate I. The lodicules at the time of blooming differ considerably from those before blooming when compared with each other. As can be seen at the first glance, the former are much more swollen than the latter and the part that swells is the lower part which is of yellowish brown colour. The swelling goes on outward (towards the lemma) while the back and upper parts do not swell so much.

There is one thing which requires special attention with regard to the lodicules at the time of blooming. When the lodicules are swollen to the fullest extent, cracks are produced. Figure 7 in Plate I shows the lodicule with a crack in it.

As shown in the Figure, a crack is made across the front edge as if it were cut by a knife. Cracks are also sometimes produced in other parts of the lodicules. Such cracks are not made on the days when the environmental conditions are not favourable. Fluid is secreted from these cracks. This fact led the writer to have doubt whether the appearance of cracks has anything to do with the closing of flowers and led him to study this point more in detail.

(iii) Forms of lodicules of oats after closing

The form of the lodicules after the closing of a flower is shown in Fig. 8, Plate I. The part which swells most when blooming occurred sinks and two or three folds are made horizontally.

TABLE 30. *The size of lodicule*

(Averaged value of 50 Lodicules)

	4½ hours before blooming	1½ hours before blooming	Beginning of blooming	1 hour after blooming	1 hour after flower closing
Length (mm.)	2.1645	2.1647	2.0702	1.9600	1.9290
%	100	100	96	91	89
Width (mm.)	0.7845	0.8231	1.4[illegible]95	1.0736	0.9741
%	100	105	182	137	124
Thickness (mm.)	0.4598	0.5081	0.8635	0.6690	0.5335
%	100	111	192	146	116

TABLE 31. *Relation between the changes in the form of lodicules and temperature on July 4, 1930*

Time	9.00 a.m.	10.00 a.m.	11.00 a.m.	12.00 m.	1.00 p.m.	1.30 p.m.	1.45 p.m.	2.00 p.m.	2.30 p.m.	3.00 p.m.	3.30 p.m.	4.00 p.m.	4.30 p.m.
Temperature (C.)	18.0°	18.8°	19.8°	20.2°	20.6°	20.8°	21.0°	20.6°	19.7°	19.4°	19.0°	18.9°	18.8°
Length (mm.)	—	2.1750	—	2.1825	—	2.1825	—	—	2.0550	2.0700	1.9200	—	1.8525
Width (mm.)	—	0.7725	—	0.7800	—	0.8175	—	—	1.3875	1.1100	0.9300	—	0.8325
Thickness (mm.)	—	0.4500	—	0.4800	—	0.4875	—	—	0.9150	0.7500	0.5925	—	0.5025

(Averaged 10 Lodicules)

TABLE 32. *Changes in the weight of lodicules before and after the time of blooming in 1930*

	4½ hours before blooming	1½ hours before blooming	Beginning	1 hour after blooming	1 hour after the flower closing
Weight (mg.)	0.49	0.53	1.07	0.82	0.58
%	100	108	218	167	118

(Averaged 50 Lodicules)

The foregoing statement deals only with the outer forms of the lodicules before, during and after blooming respectively. Table 30 shows the measurements of the lodicules at each stage of blooming.

Table 30 indicates that prior to blooming almost no changes can be recognized in length and when blooming begins the lodicule has a tendency to become shorter by degrees. Suppose the length of the lodicule $4\frac{1}{2}$ hours before blooming is 100 in ratio, then at the beginning of blooming it becomes 96 and when it closes the length becomes 89. Neither the width nor thickness show any marked difference before blooming, but when blooming occurs they increase rapidly and nearly double in measurement.

In Table 31 the changes in the form of the lodicule as mentioned above are correlated with temperature.

As shown in Table 31, the changes in the form of the lodicule do not occur while the temperature rises, and there is hardly any difference recognizable between the sizes of the lodicule at 1:30 p.m., and those at 12 a.m. and at 12 m. But when these sizes are compared with those of lodicules of the flowers beginning to bloom at 2:30 p.m., there is a remarkable difference in form. Therefore the changes in size seem to take place rapidly between 1:30 p.m. and 2:30 p.m., and the highest degree of temperature on this day was registered at 1:45 p.m. Therefore it seems that the changes in size occur rapidly when the temperature begins to lower from its highest point. This is made clear when we observe the changes in the matter contained in the lodicule as related in section 4.

Table 32 gives the results of the comparison of weight as perceived while the changes are going on.

As shown in Table 32, in weight, too, there is hardly any difference recognizable before blooming, but as soon as the flower begins to bloom, the lodicule increases rapidly in weight. When compared with that $4\frac{1}{2}$ hours before blooming, it becomes more than twice as heavy.

4. Chemical Changes in Lodicule of Oats

As mentioned in the preceding section, the lodicule of oats undergoes a remarkable change in its external aspect when booming takes place. This fact easily suggests that some chemical changes may occur in its contents. The writer, therefore, conducted the following experiments in order to find out how the two qualities, quantity of reducing sugar and the action of diastase, differ before blooming, during blooming

and after closing respectively, and the relation between these chemical changes and the changes of temperature.

Materials used for these experiments were the same as those used in the experiments described in Section 3.

(i) Quantity of reducing sugar in lodicule

ZUDELLEL,[42] NAKAO[30] and others have studied on the sugars contained in lodicule-cells of cereals and reported that the swelling of the lodicule is due to the tremendous increase of turgidity which is caused by these sugars.

NAKAO stated that the materials which cause turgidity are sugars that reduce Fehling's solution, the chief sugar being glucose.

The writer experimented to find out what changes occur in the quantity of reducing sugars (chiefly glucose) contained in the lodicule at the time before, during and after blooming respectively and also to find whether there is any relation between temperature and these changes.

Materials and Methods

The writer made an experiment on the qualitative reaction of reducing sugars by using Benedict's reagent[31] of sugar. He put two crushed lodicules at each time on a slide and poured two drops of reagent on them and then heated. When reducing sugars were present a reddish yellow deposit was formed.

In this case, of course, the quantity of reducing sugar can not be shown, but the degree of deposition can be shown by the number of positive signs. Table 33 shows the results of this experiment.

As shown in Table 33 the fact that a small quantity of deposit is formed before blooming indicates that there exists only a small quantity of reducing sugars (glucose). As the hour of blooming approaches, the quantity of deposit increases by degrees and at the time when blooming takes place its deposit increases in great quantity. And after the blooming the deposit increases more and more as time elapses until the greatest quantity of deposit is formed just before and after closing. The period of deposit formation before the time of blooming is after the highest temperature of the day is reached.

In the above experiment the reducing sugar contained in the lodicule was detected by means of reagent and therefore the reducing sugar at each period could not be compared quantitatively with one another. The writer, therefore, in order to find out the quantity of reducing

TABLE 33. *Results of experiments on the qualitative reaction of reducing in the lodicules of oats before, during and after blooming in 1930*

Date \ Time		8.30 a.m.	9.00 a.m.	9.30 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 a.m.	1.00 p.m.	1.30 p.m.	2.00 p.m.	2.30 p.m.	3.00 p.m.	3.30 p.m.	4.00 p.m.	4.30 p.m.	5.00 p.m.	5.30 p.m.	Time of blooming
June 30	T(1)		23.4		25.1		25.4		26.6		25.0		25.0		24.5			24.3		24.0	2.00 p.m.
	S(2)		+		+		+		+		++		++++		++++			++++		+++	
July 1	T	20.0		22.2		23.0		23.5	23.8	23.3	23.2			22.0		21.0	20.5		20.0		2.30 p.m.
	S	+		+		+		+	+	+	+			++++		+++++	+++++		++++		
July 2	T	22.0		23.0		24.0		25.2		25.2	27.8	26.4	26.4	27.6	26.4	25.8	26.0	25.8	25.4	25.0	4.30 p.m.
	S	+		+		+		+		+	+	+	+	+	++	++	++	++++	+++++	++++	
July 3	T	19.4		21.8		22.8	23.4	22.9	22.6	22.8	22.5	20.4		19.8	19.6		16.8			15.0	1.30 p.m.
	S	+		+		+	+	+	+	+	++	++++		+++++	+++++		++++			++++	
July 4	T		17.6		19.0		17.8		19.8		20.0		19.5		19.8	19.2		18.6		18.0	2.00 p.m.
	S		+		+		+		+		+		++++		+++++	+++++		++++		++++	
July 5	T		19.8		20.0		21.0		21.3		21.3		21.8	21.0	20.8			19.8		19.0	2.30 p.m.
	S		+		+		+		+		+		+	++++	+++++			++++		++++	

(1) T = Temperature (C.)
(2) S = Degree of deposit.

sugar made use of the "Beltrand" method, using 100 lodicules at each period. As glucose is the chief constituent of reducing sugar, the writer taking the whole reducing sugar found in this way as glucose, determined its quantity in one lodicule at each period. Table 34 shows the results thus obtained.

TABLE 34. *Result of quantitative analysis of reducing sugar in a lodicule* of oats obtained before, during and after blooming period in 1930*

	Before blooming (2–3 hours)	In the process of blooming	After blooming (1–1½ hours)
Glucose (mg.)	0.0147	0.0673	0.0392
%	100	450	260

* (An average of 100 lodicules)

As shown in Table 34, the quantity of glucose contained in the *lodicule is the greatest in the process of blooming. It is 4.5 times as* great in comparison with the quantity of glucose before blooming. The quantity decreases after the closing of a flower, but remains 2.6 times that contained before the opening of a flower.

(ii) Action of enzyme

It was mentioned in the preceding paragraph that at the time of blooming the sugar quantity increases enormously in the lodicule. The fact that sugar increases in this way makes it necessary to investigate the manner of diastase action on starch in the lodicule in connection with the formation of sugar. Therefore the writer experimented in the following manner on the action of diastase in the lodicule before, during and after blooming.

Methods

Two crushed lodicules were put in a watch-glass and two drops of 0.02% solution of soluble starch were added and this was covered with another watch-glass of the same size. The glass was kept in a thermostat at 30°C for one hour and then taking it out $N/10$ of j. j. Kali solution was added. If any starch is contained in it, an alkaline reaction results, but when starch is saccharified by enzyme this reaction does not take place.

The writer investigated whether there is any action of diastase in the lodicule by means of this method, the following being the results.

TABLE 35. *Result of experiment on the action of diastase in the lodicules of oats obtained before, during and after blooming in 1930*

	Before blooming				In the process of blooming		After blooming
	More than 3 hours	3–1½ hours	1½–½ hours	½ hour	*Blooming* flower	*Non-blooming* flower	After closing
Jul. 25	− − −	− − −		+ + +	+ + +	− − −	+ − −
„ 26	− − −	− − −		+ + +	+ + +	− − −	− − −
„ 27		− − −	− − +		+ + +	− − −	− − −
„ 28	− − −				+ + +	− − −	+ − −
Aug. 1	− − −		− − −		+ + +	− − −	− − −
„ 2	− − −		− − −		+ + +	− − −	+ − −
„ 3		− − −		− − −	+ + +	− − −	− − −
„ 20		− − −			+ + +	− − −	− − −
„ 22		− − −		+ − +	+ + +	− − −	− − +
„ 23	− − −	− − −			+ + +	− − −	− − −

(−) signifies negative results as to the action of diastase and (+), positive.

Table 35, shows that the action of enzyme is negative in the majority of those lodicules before blooming and that it is positive in every one of those during blooming, that is, starch has already been saccharified. The action is negative in most cases after the closing of the flower. At the same time an immature spikelet which was to bloom the next day was taken and the action of diastase in it was investigated, the results being that every one of them was negative.

Again, there are lodicules which show positive action of enzyme even before blooming, and when considered in connection with the changes of temperature of the day, there is shown a reaction of starch before the highest temperature is reached, but when the temperature grows lower no starch reaction is shown.

From this fact it can be considered that the action of enzyme commences when the temperature begins to decline.

5. SUMMARY

1. The opening and closing of the stomata are in a parallel relation with temperature and humidity. Blooming occurs when the stomata begin to close.

2. When a head is put in a bottle containing some water, blooming

always takes place, but when it is put in a bottle containing no water and is exposed to the air, blooming never takes place. But when the latter is put in a glass jar and is cut off from the outer air, blooming takes place. That is to say, a special supply of water is not necessary for blooming, but only so much water is needed as is sufficient to keep the heads alive.

3. Almost no changes take place in the form of the lodicule before blooming.

4. Extraordinary changes occur during blooming. The lower half of the lodicule swells considerably.

5. Its decreases during blooming, but both its width and thickness increase about double.

6. Its weight which was 0.49 m.g. before blooming becomes 1.07 m.g., about double.

7. Such changes take place when the temperature begins to go down from its highest point of the day.

8. Reducing sugars exist in a very small quantity before opening occurs, and do not increase up to a certain period of time. The quantity, however, goes on increasing from the time immediately before the flower opens and when blooming occurs it increases rapidly until it attains about 4.5 times the quantity Present before blooming.

9. The action of enzyme is hardly recognizable before opening, but the action takes place actively immediately before opening. The maximum is reached during blooming and no action is recognized after closing of the flower.

10. The changes of reducing sugar and enzyme action have intimate relation with temperature. When temperature is rising, no change takes place in reducing sugar nor is any enzyme action recognized. But when temperature goes down and reaches a certain point, the reducing sugar increases, and the action of enzyme appears remarkably.

Discussion

Which element of all outside conditions has the closest influence upon the blooming of a plant varies according to the different kinds of plants. Each investigator has a different opinion upon this question. A. D. Virville and F. Obaton (1925) made a biologic study on the opening of some flowers and methodical observations made under natural conditions have led them to the conclusion that the movements of the

corolla, particularly in the case of ephemeral flowers, are dependent upon the variable meteorology of the day, more particularly upon variations in temperature. It was shown that temperature is the only agent that determines the movements of the petals as well as their fall and accordingly blooming does not take place during rainy or cold weather. It was also shown that each different species has a different time of blooming, because its flower begins to bloom at a different degree of temperature. After experimenting on the relation between blooming and illumination, they state that illumination apart from temperature has no important influence on blooming except that it has some relation with the nutriment of a plant. They state also that the moisture has very little to do with blooming. GOLDSMITH (1925) who studied the cause of opening and closing movements of a flower reported that in some flowers they are influenced by the changes in temperature, but in others by the changes in illuminating power. Low temperature makes some flowers close and high temperature has the same effect on others.

In summarising various opinions regarding the relations of these meteorological factors to cereals, most investigators are of the opinion that the most important factors are light, temperature and humidity and that pressure, wind and other factors have no direct relation to it. Investigators differ in their opinions as to which of these factors, light, temperature and humidity, is the most important of all.

KÖRNICKE (1885) observed that the blooming of cereal flowers occurred in a small number on a clear day and came to the conclusion that strong sunshine hinders the opening of flowers. AKEMINE (1914) stated that light has no direct connection with the blooming of rice flowers. NAKAO (1911) stated that a part of weak reflected rays help flowers to bloom. NOGUCHI (1929) stated that orange rays urge flowers to bloom sooner than others followed by yellowish rays, and that it is due to their stimulation that flowers are urged to open. This stimulation is felt by the organization in the ovary and the swelling of the lodicules that has a direct relation with the opening of flowers is caused by the transmission of this stimulation.

C. E. LEIGHTY (1924) observed that most of the wheat flowers under observation bloomed in daylight, but flowers kept in a dark room bloomed completely and in the same manner as those under natural conditions. These facts led him to conclude that light is not essential for blooming.

T. K. WOLFE (1925) observed the blooming of orchard grass and found that of the 5861 flowers observed in the process of blooming,

76.9% bloomed from sunrise to noon, 6.6% from noon to sunset, 0.3% from sunset to midnight, and 16.3% from midnight to sunrise and he also denied the indispensibility of light to blooming.

The present writer observed the relation between the blooming of oat flowers and daylight and came to the conclusion that the time of blooming in the day and the opening of flowers do not depend on the number of hours of daylight. He also succeeded in causing flowers to bloom under favourable conditions of temperature even when light was shut out or when they were placed in a dark room. The writer found as reported in another paper[21] that *Avena strigosa*, *Avena balbata* and *Triticum aegilopoides* under observation in Sapporo did not bloom during daylight, but opened after sunset, from about 8 to 9 p.m. Now his further observations and experiments recounted in this paper show that light has nothing to do with the blooming of *Avena sativa* also. The blooming of oat flowers occurs in rainy weather just like that of rice flower does as reported by AKEMINE (1914). This fact shows that rain itself does not hinder the blooming of flowers.

KÖRNICKE (1885) states that a shower on a fine day hastens blooming, and the writer also observed this fact often in the process of his experiments. When water was poured on the panicle from a watering pot, the opening of flowers occurred from two to three hours earlier than those under natural conditions. This is considered to be due to the fall of temperature caused by pouring the water and not to the mechanical reaction of the flower thereto.

KOBAYASHI (1925) states that as the blooming of rice flowers occurs when humidity is decreasing, the variation of decrease of humidity may have some relation to blooming.

In the writer's observations, in oats just the opposite result to NOGUCHI's was obtained, that is, when humidity was increasing, blooming occurred. Furthermore the writer succeeded in bringing about the blooming of oat flowers by changing temperature only and not humidity. From this fact it is to be concluded that humidity has no direct relation to blooming, but humidity variations are only secondary results caused by the changes in temperature.

It is generally considered that temperature is one of the important external factors that affect blooming. KÖRNICKE (1885) states that during night and morning when temperature is low the blooming of wheat flowers is delayed.

RIMPAU (1882) states that the intensive blooming of wheat flowers

of a day depends upon the temperature of early morning. There are, however, very few investigators who discuss what action of temperature is related to blooming. AKEMINE (1914) alone touches upon this problem. He states that blooming has relation not merely to the temperature at the moment of its occurrence, but that it occurs after flowers are exposed to the temperature higher than a certain degree for some time. That is to say, blooming does not result from the momentary action of temperature, but it can be considered to be the result of continuous action of temperature.

The writer observed occasionally in oats that, when the temperature of the day was within the range of temperature for blooming or when it was the optimum, no blooming occurred in natural conditions. He also observed that even in the thermostat which maintains the optimum temperature oat heads did not bloom through an entire day. From these facts the influence of temperature upon blooming can not be considered to depend on only the high or low degrees of temperature nor on continuous optimum temperature. According to the writer's investigations, oat flowers bloom when temperature is falling from the maximum of the day and they never open when temperature is rising. Even when blooming began to occur after the fall of temperature, if it rises again, the rest of the flowers stop blooming for a time and when temperature begins to fall, the flowers again begin to bloom. Therefore the time of blooming within a day depends on the variations of temperature. It varies also considerably according to the day, the earliest being 12:45 p.m. and the latest 5:45 p.m. That is to say, on the days when the highest temperature is reached comparatively early, the time of blooming is also early, and on the days when the highest temperature is reached later, the time of blooming is also later. When the variations of temperature are slight, no blooming occurs. From the results mentioned above, it can be considered that the influence of temperature on blooming is derived from its variations.

NAKANO (1929) and ISEDA (1930) observed that in rice flowers most blooming occurred when temperature was rising and a little occurred when temperature was falling. Just the opposite conditions of temperature are considered to encourage blooming of oat flowers. There seems to be a difference in sensibility to these temperature variations between oats and rice, the former depending on the decrease of temperature and the latter on the increase thus causing difference in the time of blooming. Further studies may solve this problem.

As has been discussed above the writer ascertained that blooming of oat flowers depends mostly upon temperature, especially its decreasing variations. Therefore the writer desires further to discuss what the action of the variation of temperature has to do with the physiology of blooming.

HACKEL (1881), RIMPAU (1882) and AKEMINE (1914) have already proved that the swelling of lodicules is the direct cause of blooming not only in cereals but also in many graminaceous plants. The writer *also studied on the morphological change of the lodicules of oats at the* time of blooming and came to the same conclusion. ZUDELLEL (1910) studied regarding the physiology or swelling of the lodicules and he proved that the lodicule-cells contain highly permeable materials. NAKAO (1911) states that the swelling of the lodicule is caused by the marked increase of turgidity. This increase of turgidity is caused by the change of disaccharoid into mono-saccharoid in the lodicule cells. The closing of flowers is caused by the protoplasmic membrane of the lodicule cells degenerating into permeative nature and the sugars which caused swelling are sent out of the cells with the consequent shrinkage of the lodicule and the closing up of the elastic outer glumes.

There has, however, been no one who has tried to discuss the cause which brings forth the change in the matter contained in the cells of the lodicules. The writer has observed that the variations of temperature affect blooming directly. From this fact it can be considered that the swelling of the lodicule has an intimate relation with the variations of temperature. According to the writer's experiment, the lodicules do not swell while the temperature is rising, but they begin to swell when the temperature starts to lower from the highest point reached on the day. At the time of blooming the swelling reaches its maximum. Considering from the above fact it is clear that the cause which brings forth the change of the matter in the cells of the lodicules is variation of temperature. NAKAO (1911) states that glucose is one of the chief substances which cause the swelling of the lodicules in rice flowers. The writer conducted experiments on sugars in the oat lodicules, especially on reducing sugar (glucose), in the cells before and after blooming and ascertained that when temperature begins to lower *from its highest point of the day, glucose begins to increase and at the* time of blooming it increases suddenly. This fact coincides with the morphological variations of the lodicules. From the fact that at the time of blooming the sugars increase rapidly in the cells of the lodicule

it can be easily imagined that action of enzyme is in progress in this case. According to the writer's experiment on this point, just before blooming, when temperature begins to come down from its maximum point, the action of enzyme makes its appearance. The action is most remarkable at the time of blooming.

Conclusion

The blooming of oat flowers, as recognized by many investigators, takes place in the afternoon, but the time of blooming differs considerably according to the day, the earliest being 12:40 p.m. and the latest 5:45 p.m. There are, however, some cases in which uo blooming takes place throughout the whole day even in the flowering period. These facts are considered, according to the observations, to be due to various meteorological factors particularly to the variations of temperature and of humidity. Blooming never occurs before the temperature reaches the highest degree of the day, but it occurs during its fall from the highest point.

The range of the highest temperature during the day, in the writer's observation, is 32.8–15°C.; that of the temperature at the blooming time is 29.8–14°C.; the fall of temperature differs according to the meteorological condition of the day, but the difference between the highest temperature and the temperature at the time of blooming is 0.2–7.5°C.; *the blooming time is 16–285 minutes after the hour of the highest tem*perature. Blooming occurs during the increase in humidity which is caused by the fall of temperature.

The writer has made various stndies in order to determine which of these causes of blooming, the variation of temperature or that of *humidity, is the more effective and direct one.*

He at last succeeded in controlling both blooming time and blooming itself by means of the artificial adjustment of temperature. He failed, however, in controlling them by adjusting humidity.

This fact has led the writer to the conclusion that the most effective *external factor which controls the blooming of oats is temperature.* To cause blooming, the fall of temperature which has risen to the above mentioned range is necessary and with this the humidity increases and the stomata show a tendency of closing themselves. Therefore it can be considered that blooming takes place when it becomes unnecessary for a plant to take in a great quantity of water.

The chemical changes of the lodicule cells that are necessary for their swelling at the time of blooming do not take place while temperature is rising, but they take place during the fall of temperature from its highest degrees. Therefore the action of temperature on blooming is not caused by high or low temperature, but by its variations in the day, especially by the decrease of temperature after the time of its maximum. The fall of temperature causes the change of the matter in the lodicule cells and water is accumulated in them, the result being that the lodicules swell up and blooming takes place.

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EXPLANATION OF PLATE 1.

(*Avena sativa* L.)

Fig. 1.	Floral organ.	o, ovary a, anther	s, style l, lodicule	f, filament

Fig. 2. *Cross section of the spikelet.*

d, outer glume	v, inner glume
l, lodicule	f, filament
o, ovary	

Fig. 3A. Front view of the lodicule before blooming.

Fig. 4A. Back view of the lodicule at the time of blooming.

Fig. 5A. Front view of the lodicule at the time of blooming.

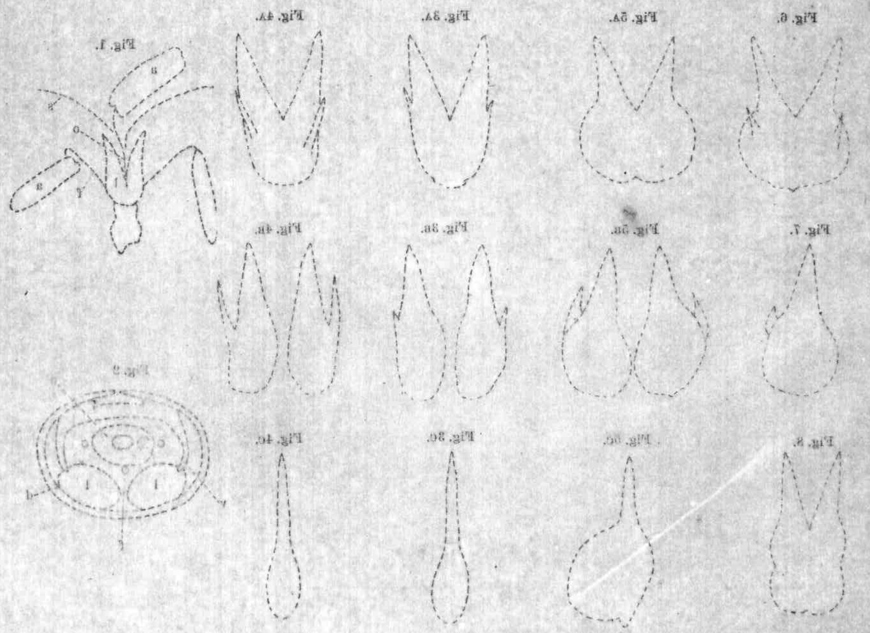
Fig. 6. Back view of the lodicule at the time of blooming.

Fig. 7. Lodicule with a crack in it.

Fig. 8. Front view of the lodicule after the closing of a flower.

B shows the lateral view of A and C the dorsal view of B.

PLATE I



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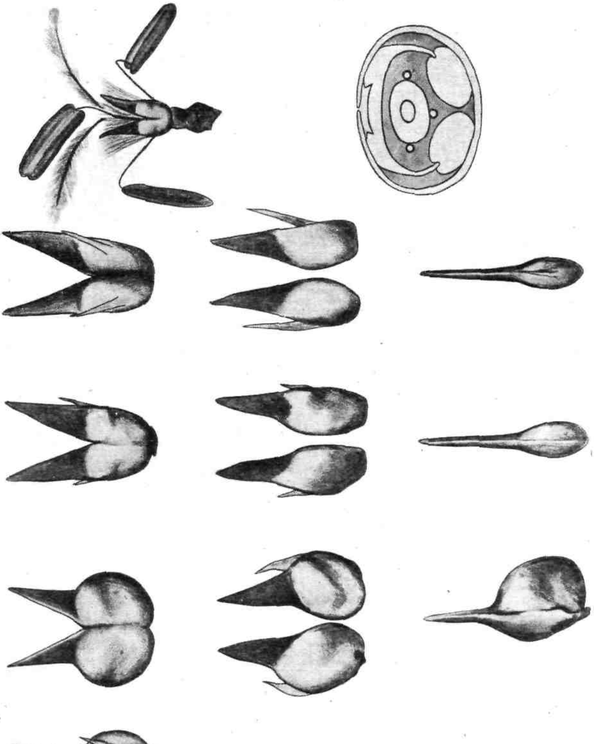

APPENDIX

APPENDED

(Number of experi

Date		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 p.m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Jul. 11, 1927	T.(1)	—	26.9	25.8	28.2	28.3	28.3	28.1	28.5	28.0	28.2	**29.0**	29.0	28.5
	H.(2)	—	—	—	—	—	—	—	—	—	—	—	—	63
	B.(3)													
„ 12, „	T.	—	—	27.0	27.2	27.4	27.8	28.4	27.8	28.2	28.9	28.2	27.8	28.4
	H.	—	—	68	72	72	69	69	69	69	69	69	72	72
	B.													
„ 13, „	T.	25.2	24.6	26.6	26.2	**27.0**	25.0	25.4	24.9	24.1	23.6	23.6	23.2	23.0
	H.	78	82	75	82	72	78	82	82	86	90	90	90	90
	B.												*17*	*24*
„ 14, „	T.	21.1	19.8	22.2	**23.0**	23.0	22.8	22.4	21.4	22.4	20.8	20.1	20.6	20.9
	H.	85	100	95	90	90	90	90	90	90	89	95	90	90
	B.												*1*	*1*
„ 15, „	T.	19.6	21.1	22.4	24.4	24.8	25.8	**26.6**	24.2	23.6	24.6	24.0	24.2	24.8
	H.	100	95	100	82	86	86	86	86	86	90	86	90	86
	B.													*12*
„ 16, „	T.	24.8	26.6	27.2	27.0	28.2	28.1	27.4	27.8	28.2	28.4	29.8	**30.0**	29.8
	H.	86	79	75	75	75	72	75	75	75	75	73	70	67
	B.													
„ 17, „	T.	22.6	23.2	**25.4**	25.2	25.1	25.4	25.4	25.0	24.2	24.2	24.6	24.4	24.4
	H.	100	95	86	86	90	86	86	90	90	90	90	90	95
	B.													*3*
„ 18, „	T.	19.8	20.4	20.7	20.5	20.6	21.0	20.8	21.0	21.0	21.2	21.4	21.4	**21.7**
	H.	95	100	95	95	100	100	100	100	100	100	100	100	100
	B.													
„ 19, „	T.	20.6	22.0	22.2	22.1	22.6	24.6	24.6	**24.8**	23.0	23.9	23.2	23.1	23.8
	H.	100	90	90	95	90	95	82	82	82	74	81	81	82
	B.										*29*	*12*	*89*	*36*
„ 20, „	T.	20.2	21.4	**24.5**	24.0	23.8	23.7	23.4	23.5	23.2	23.2	22.9	22.9	22.4
	H.	89	85	78	74	78	82	77	81	81	81	81	81	81
	B.													
„ 21, „	T.	20.6	22.0	23.0	**23.2**	23.0	22.9	22.9	22.9	22.9	22.9	22.9	22.8	22.6
	H.	100	90	86	85	81	90	86	86	86	86	86	86	86
	B.													
„ 22, „	T.	21.0	24.4	25.6	26.0	25.8	25.8	24.6	24.6	25.6	26.2	26.0	**27.3**	27.3
	H.	90	86	78	78	82	82	82	82	82	78	82	79	79
	B.													
„ 23, „	T.	19.2	20.0	21.2	21.8	21.2	21.5	21.8	22.2	22.0	22.2	22.1	22.3	22.6
	H.	89	100	80	81	85	85	81	85	81	85	81	81	81
	B.													

(1)—T = Temperature (C°.) (2)—H = Humidity (%).

(3)—B = Number of flowers opened.

TABLE 1.

mental-plants 10)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
27.0 72	26.6 72	26.4 75	26.0 75	26.0 75 *22*	26.4 75 *1*	28.5 66	28.6 66	27.9 72 *20*	28.2 72 *9*	27.4 75	27.8 75	28.2 72 *8*	27.4 75 *9*	26.7 75	27.2 72	26.5 75	25.1 82
28.8 69	28.8 69	**29.0** 69	29.0 60	28.6 69	28.2 69	28.0 72	28.8 72	28.4 69	27.5 72 *15*	27.6 75 *47*	26.7 75 *17*	26.3 71 *7*	26.1 78	26.1 78	25.8 78	25.5 78	25.0 82
22.9 90 *13*	22.7 95 *3*	22.3 100 *24*	21.9 95 *6*	21.5 95 *6*	21.1 100	20.9 95	21.0 95	20.9 100	20.4 100	20.0 100	20.0 100	20.2 100	20.4 100	19.8 100	19.6 100	19.1 94	18.6 94
21.0 90	20.9 90 *20*	20.7 90 *17*	20.6 95 *12*	20.4 95	19.8 95	19.9 95	19.5 100	20.2 95	20.4 95	20.3 95	20.0 95	20.0 95	19.8 95	19.4 100	19.4 100	19.4 100	19.4 100
24.4 86 *15*	25.0 86 *90*	25.0 86 *47*	25.0 95 *3*	25.8 91 *4*	25.8 82	26.4 78	26.0 83 *17*	26.2 82 *6*	25.5 82	26.8 79	26.8 83	26.0 82	25.3 82	25.2 86	25.2 86	25.0 82	24.5 82
29.0 72	29.4 69	27.0 79 *1*	26.3 75 *30*	26.2 82 *21*	25.4 82 *31*	25.2 82 *21*	24.9 86 *12*	24.1 90 *9*	23.4 90 *7*	22.8 90	22.8 90	23.0 90	22.5 90	22.7 90	22.6 90	22.7 86	21.8 95
24.5 95 *6*	25.4 86	24.7 90 *60*	24.2 90 *14*	24.1 90 *16*	24.1 90 *11*	24.1 95 *6*	24.2 90	24.6 90	24.2 90	24.8 90	24.4 90	24.2 90	24.0 90	23.9 95	23.9 95	23.7 90	23.4 95
21.5 100	21.4 100	21.6 100	21.4 100	21.4 100	21.4 100	21.4 100	21.0 100	21.5 100	21.6 100	21.6 100	21.8 100	21.7 100	21.6 100	21.4 100	21.2 100	21.0 100	21.0 100
24.0 82 *6*	24.2 78 *5*	24.2 78	22.8 77 *48*	22.6 81 *12*	22.5 81	23.1 85 *4*	22.2 81	21.6 85	20.8 90	20.8 85	20.6 85	20.6 90	20.8 90	20.4 89	20.2 89	19.6 95	19.0 100
22.0 85 *20*	22.0 85 *40*	22.6 86 *9*	22.4 81 *20*	24.0 82	24.4 78	24.6 78	24.2 78	24.2 78 *8*	24.2 78	24.2 78	23.6 82	23.4 81	22.3 85	21.4 90	20.8 90	20.4 89	19.8 95
22.4 90	22.0 90 *20*	21.8 90 *22*	21.2 95	21.1 95 *3*	21.1 95	21.1 95	20.6 95	20.4 95	20.2 95	19.9 95	20.0 100	21.0 95	20.0 100	20.2 95	20.2 95	20.2 100	20.4 95
27.0 79	27.0 79	27.2 79	27.2 79	27.0 79	27.0 79	26.6 83 *10*	26.3 82 *9*	26.2 82 *21*	25.6 78 *11*	25.6 82 *12*	25.4 82 *4*	25.2 82	25.4 82	25.2 82	24.6 86	24.6 90	24.8 90
22.0 90	22.6 81	**23.2** 81	22.4 77	22.6 77 *3*	22.7 77	22.2 81 *2*	22.2 81	22.2 77	22.6 77	22.2 81	22.2 81	22.2 81	21.8 81	21.8 81	21.8 77	21.0 80	20.5 85

TABLE 1—

		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Jul. 24, 1927	T.	21.0	22.5	24.0	24.8	25.1	26.0	26.0	26.0	26.4	27.0	27.2	**28.0**	27.8
	H.	90	85	74	74	71	71	68	68	65	65	68	69	72
	B.													
„ 25, „	T.	21.0	23.0	23.8	25.4	26.2	26.8	27.0	27.4	27.3	27.8	28.0	27.7	**28.2**
	H.	90	86	82	74	75	72	68	68	65	69	69	66	66
	B.													
„ 26, „	T.	23.2	23.8	24.7	24.2	25.4	26.1	26.2	26.2	26.0	26.0	26.2	26.3	26.4
	H.	77	78	71	74	74	68	68	68	68	71	71	68	71
	B.													
„ 27, „	T.	24.2	25.4	25.2	25.6	26.0	26.3	26.8	26.7	27.3	27.7	27.5	27.7	**27.6**
	H.	78	74	78	78	75	75	75	72	72	66	72	72	69
	B.													
„ 28, „	T.	24.4	26.2	27.8	26.6	27.0	27.2	27.6	27.6	27.8	**28.3**	27.6	27.8	27.2
	H.	82	78	72	72	68	68	69	69	66	66	69	69	65
	B.													
„ 29, „	T.	23.2	24.0	24.3	25.6	25.6	27.1	26.8	27.0	27.0	**27.3**	27.0	26.5	27.0
	H.	86	82	74	75	68	72	65	68	65	65	62	68	68
	B.													
„ 30, „	T.	25.4	25.4	26.2	27.2	27.2	28.2	28.8	**29.2**	28.7	29.0	28.8	28.6	28.4
	H.	71	71	71	68	68	66	66	66	66	66	69	69	69
	B.													
„ 31, „	T.	22.8	24.4	25.2	24.6	24.6	24.6	24.6	**25.2**	25.0	24.6	24.8	24.4	24.4
	H.	95	90	86	82	82	86	82	82	82	82	82	82	86
	B.												2	5
Aug. 1, „	T.	21.8	23.2	25.6	26.0	26.6	26.4	27.6	27.3	27.2	27.6	28.0	27.7	27.9
	H.	95	95	86	82	79	82	75	75	75	75	79	75	75
	B.													
„ 2, „	T.	26.4	27.2	**28.6**	28.4	28.2	28.2	27.8	27.8	27.8	27.6	27.6	27.6	28.4
	H.	78	75	69	69	72	72	75	75	72	75	75	75	72
	B.													
„ 3, „	T.	25.4	25.4	28.2	26.6	25.4	25.0	25.8	26.4	26.4	27.0	28.6	**29.0**	28.4
	H.	100	95	79	87	90	90	91	86	91	83	83	79	75
	B.													
„ 4, „	T.	25.6	26.4	27.1	27.0	27.2	27.4	27.8	27.8	27.6	28.2	28.4	28.5	**28.6**
	H.	82	82	79	75	75	75	75	69	72	69	69	69	72
	B.													
„ 5, „	T.	25.2	26.8	27.8	28.6	28.4	28.2	28.4	28.8	**29.0**	28.2	27.9	27.6	27.8
	H.	86	83	75	72	72	75	72	72	72	72	76	75	75
	B.													
„ 6, „	T.	26.6	26.4	25.6	25.6	25.4	26.4	**26.8**	26.8	27.0	26.8	26.6	26.4	26.2
	H.	83	82	82	82	82	82	83	83	83	79	83	82	82
	B.													

(*Continued*)

1.45 m.p.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
26.6 72	26.8 72	26.6 72 *4*	26.4 65	25.9 68 *9*	25.4 67 *27*	25.6 72 *2*	25.8 71 *22*	24.8 71 *4*	24.6 74 *1*	25.0 74	24.2 78	24.2 82	24.0 82	23.0 86	23.4 86	23.2 81	22.8 86
27.6 69	27.5 68	27.3 75	27.0 72	26.5 71	26.7 72	27.2 72	27.5 72	27.3 72	26.8 75	26.4 75 *21*	25.5 74 *36*	25.2 78 *8*	25.2 78	24.6 86	24.3 86	24.1 86	23.9 90
26.6 68	26.8 68	26.9 68	**27.0** 68	27.0 68	26.8 68	26.6 72	26.2 71 *8*	26.4 75 *29*	25.4 74 *9*	25.2 74 *12*	25.2 74	25.0 78	24.8 74	24.3 78	24.0 78	23.8 82	23.5 78
27.6 72	**28.0** 72	27.4 62	27.5 68	27.2 72	26.8 72 *12*	26.5 71	27.0 78 *5*	26.2 75	26.4 75 *13*	26.6 75	25.6 78 *1*	25.4 78	25.4 82	25.4 82	25.4 82	25.4 82	25.0 82
27.0 68	26.7 72	26.5 75	27.0 68	26.8 71	26.4 75 *7*	27.0 68	26.8 68 *2*	26.8 72 *6*	26.4 71 *4*	25.8 71 *5*	25.6 75 *2*	26.0 75	25.4 74	25.4 78	24.6 78	24.4 78	24.6 82
27.0 68	27.4 68	27.0 62	26.2 65	26.8 68 *9*	26.4 71	26.2 71 *5*	26.0 68 *4*	25.8 71 *5*	25.5 74 *1*	25.5 74	24.8 78	25.0 74	24.9 78	24.2 78	24.2 82	24.0 78	23.2 90
27.8 69	27.8 69	27.6 72	27.6 69	27.4 72	27.4 68	27.0 72 *4*	26.4 71 *8*	26.3 75 *5*	26.0 75	25.4 78 *7*	24.8 78 *3*	24.4 82 *3*	24.4 78	24.6 78	24.4 78	24.4 78	22.2 100
24.6 82 *2*	25.2 78 *2*	25.2 82 *3*	24.8 82 *2*	25.0 82 *1*	25.0 82	24.5 82	23.8 86	23.6 86	23.6 86	23.4 86	23.4 86	23.0 90	22.6 90	22.6 90	22.6 90	22.2 90	22.2 90
27.6 72	28.0 69	28.0 69	28.5 69	**29.0** 66	28.6 69	28.2 69	28.6 69 *6*	27.8 63	27.8 72 *2*	28.6 72 *10*	26.9 75 *6*	26.5 78 *13*	25.6 78 *1*	25.4 82	25.0 82	24.8 82	24.4 82
28.3 75	28.0 72	27.8 75	27.6 75	27.0 75 *12*	26.2 78 *7*	26.3 82 *1*	26.0 86	25.8 86 *12*	25.8 91	25.8 91 *1*	25.4 95	25.0 100	25.0 100	24.8 100	24.8 100	24.6 100	24.2 100
28.0 79	28.4 79	28.4 79	29.0 76	28.6 76	28.8 76	28.6 76	27.2 79 *13*	26.8 79 *6*	26.4 82 *5*	26.4 82	25.2 90	25.2 95	24.8 95	24.8 90	24.2 95	23.8 95	23.4 95
28.6 72	28.6 69	28.4 69	28.0 72	27.8 72 *5*	27.8 72 *2*	27.5 72 *1*	27.3 72 *4*	27.2 75 *4*	27.0 72	26.6 75 *1*	26.4 75	26.2 75	25.9 78	25.9 78	25.0 82	24.6 86	23.2 90
27.8 75	27.8 75	27.8 75	27.6 75	27.6 75	27.2 79 *6*	27.0 82 *3*	26.5 82	26.2 86 *3*	26.0 86 *3*	26.0 86	26.0 86	25.4 86	25.6 86	25.2 86	25.0 86	25.0 86	25.0 86
25.8 82	26.0 82 *2*	26.0 82 *4*	25.8 82 *3*	25.6 82 *3*	25.0 82 *2*	24.6 86 *2*	24.8 82 *1*	24.6 82 *2*	24.2 82	24.2 82	23.8 86	23.6 86	22.8 95	22.6 95	22.6 95	22.6 90	22.4 90

APPENDED

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		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Aug. 17, 1927	T.	—	—	—	—	25.4	25.6	26.1	26.5	26.4	26.6	26.4	**26.8**	26.6
	H.	—	—	—	—	78	78	78	78	75	75	75	75	75
	B.													
„ 18, „	T.	23.6	24.2	23.6	23.6	24.2	24.2	24.4	24.6	24.8	**25.0**	25.0	25.0	25.0
	H.	90	82	86	90	86	86	82	82	82	82	82	82	82
	B.													
„ 19, „	T.	24.0	25.4	26.4	27.2	26.6	27.8	27.6	28.2	27.6	27.9	28.3	28.4	29.3
	H.	90	82	82	75	83	79	79	75	75	75	72	72	72
	B.													
„ 20, „	T.	22.2	23.8	25.6	26.3	26.2	**27.2**	26.8	26.6	26.6	26.4	26.4	26.9	26.8
	H.	85	70	68	65	68	62	65	65	65	62	65	72	68
	B.													
„ 21, „	T.	22.0	24.6	25.8	26.6	26.8	27.0	27.6	27.6	27.4	27.6	27.8	28.0	28.0
	H.	81	74	71	68	68	62	66	66	59	62	63	63	63
	B.													
„ 22, „	T.	23.0	24.2	24.8	24.4	24.8	26.2	**26.8**	25.4	24.8	24.8	24.6	24.8	25.0
	H.	90	86	82	90	86	78	79	82	82	80	86	86	82
	B.													
„ 23, „	T.	23.0	23.0	24.7	24.8	24.6	24.6	26.2	26.6	**27.4**	26.2	26.0	25.8	26.1
	H.	77	77	71	71	78	75	71	65	65	65	68	68	71
	B.													
„ 24, „	T.	18.4	18.4	18.2	18.2	18.2	19.0	19.0	19.0	19.2	19.0	19.2	19.4	19.4
	H.	94	100	100	100	100	100	100	100	100	94	100	100	100
	B.													
„ 25, „	T.	21.8	22.1	21.6	23.4	23.8	23.6	24.4	24.4	**25.0**	24.4	24.6	24.2	24.3
	H.	85	85	90	81	82	82	78	82	74	78	78	82	78
	B.													
„ 26, „	T.	20.6	21.8	23.4	24.0	24.8	25.4	25.4	25.4	25.6	25.2	25.2	25.6	**25.8**
	H.	100	100	81	78	74	71	71	71	71	74	71	71	68
	B.													
„ 27, „	T.	19.6	20.0	20.4	20.6	20.4	23.6	22.0	22.6	22.8	23.0	23.0	23.2	23.4
	H.	100	100	100	100	100	95	90	90	90	90	90	86	81
	B.													
„ 28, „	T.	20.6	20.8	22.4	23.0	23.8	23.6	23.4	23.0	23.6	23.7	23.7	24.0	**24.2**
	H.	100	90	85	77	74	73	73	73	74	78	74	74	74
	B.													
„ 29, „	T.	20.2	21.0	22.0	22.8	23.0	23.4	**23.8**	23.2	22.6	22.6	22.8	22.8	23.2
	H.	85	76	77	73	69	73	74	73	73	73	77	81	73
	B.													
„ 30, „	T.	19.2	18.4	18.6	19.2	19.2	19.0	18.8	18.8	19.6	19.6	19.6	**19.8**	19.4
	H.	100	100	1.00	100	100	100	100	100	100	95	100	100	100
	B.													

TABLE 2.

mental-plants 55)

1.45 p. m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
26.5 75	26.6 72	26.6 72	26.6 72	26.4 71	26.0 72	25.9 71 *59*	25.6 71 *16*	25.7 71 *15*	25.4 71 *43*	25.5 71 *23*	25.2 74 *13*	24.7 78 *11*	24.5 78	— —	— —	— —	— —
24.9 82	24.8 82 *47*	24.8 82 *25*	24.6 82 *9*	24.7 82 *10*	24.8 82 *4*	24.6 82 *39*	24.5 82 *14*	24.5 86 *11*	24.6 86 *4*	24.2 86 *10*	24.1 86 *2*	23.9 86 *2*	23.8 90	23.8 90	23.6 90	23.4 95	23.2 95
28.8 76	28.8 69	28.8 69	**29.6** 70	29.2 76	29.6 76	29.4 72	28.4 75	27.8 75	27.6 75 *16*	27.5 79 *22*	26.9 79 *61*	26.6 79 *61*	25.6 82 *16*	25.6 82 *6*	25.6 82	25.0 86	24.6 86
26.9 68	26.9 68	25.9 65	25.6 72 *18*	25.2 74 *51*	25.0 74 *40*	24.6 74 *12*	24.6 78 *12*	24.8 74 *5*	24.8 78 *2*	24.0 78	23.8 82	24.0 82	24.0 82	23.6 86	23.0 86	23.0 90	23.0 90
28.0 63	**28.6** 63	27.8 60	27.8 63	27.8 63	27.8 66	27.5 65	27.8 75 *8*	27.0 75 *18*	27.0 75 *15*	26.2 75 *19*	25.7 82 *9*	25.4 82	24.8 86	24.8 86	24.2 86	24.0 86	24.0 86
24.4 86	24.4 86	25.0 86	24.4 86 *44*	24.0 90 *29*	24.6 86 *18*	23.6 89 *14*	23.2 95 *16*	24.1 90 *2*	23.8 90 *2*	24.4 90	23.8 90 *1*	24.0 90	24.2 90	24.2 95	24.2 95	24.0 95	23.8 95
25.0 67	26.7 68 *1*	26.7 68	26.2 68	25.6 68 *74*	25.4 67 *54*	25.6 74 *17*	25.1 74 *26*	26.1 74 *22*	26.2 74 *4*	25.8 68	26.2 71	25.6 75	25.2 78	25.0 74	24.8 78	24.6 78	24.4 78
20.0 95	19.7 95	19.4 95	19.4 94	19.3 94	19.2 94	19.4 94	19.1 94	19.3 94	19.2 94	19.6 100	19.4 100	19.6 95	19.5 94	19.4 94	19.4 94	19.4 94	19.2 94
24.8 78	24.4 78 *2*	24.6 78 *43*	23.7 78 *96*	25.2 78 *73*	23.6 86 *43*	24.0 82 *39*	24.1 78	23.6 82	23.6 82	23.4 82	23.1 81	23.0 81	22.9 81	22.8 86	22.2 85	21.6 90	21.2 90
25.6 71	24.9 71	24.5 71	24.3 71	24.2 74	24.3 74	24.1 74 *37*	24.6 74 *18*	23.9 78 *14*	23.9 78 *4*	23.8 82 *2*	23.6 78	23.4 81	23.0 86	22.6 86	22.6 86	22.4 85	22.0 90
23.6 86	23.4 82	23.6 86	23.6 86	23.1 86	23.0 90	22.7 90	22.7 90	22.6 90 *1*	22.5 95	22.2 100	21.9 95	21.8 95	21.8 95	21.4 95	21.4 95	21.2 95	21.2 95
23.6 74	23.8 74 *23*	23.4 74 *43*	23.2 74 *44*	23.2 74 *29*	23.1 74 *46*	23.2 78 *38*	23.8 78 *26*	23.1 81 *18*	23.0 86 *6*	22.6 86 *5*	22.2 90 *1*	21.8 90	21.2 95	21.7 90	21.5 90	21.2 95	20.8 95
23.6 77	23.3 73	23.4 77	22.4 77 *2*	22.4 77 *84*	22.4 77 *30*	22.2 73 *27*	22.1 81 *10*	22.0 77 *2*	22.0 77 *1*	21.8 81	21.4 80	21.0 76	20.4 89	20.0 95	20.0 95	19.8 95	19.8 95
19.6 100	19.7 100	19.6 100	19.4 100	19.0 100	19.2 100	19.0 100	19.0 100	19.2 100	19.4 100	19.6 100	19.6 100	19.8 95	19.6 100	19.6 100	19.4 100	19.2 100	19.1 95

TABLE 2—

		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
	T.	17.8	21.0	20.8	21.4	22.6	23.4	22.4	23.2	**23.8**	22.6	22.4	22.4	23.6
Aug. 31, „	H.	60	57	66	69	72	72	80	75	76	72	55	79	76
	B.													
	T.	20.2	22.2	23.8	24.8	24.8	24.4	24.4	25.2	25.4	**26.0**	26.0	25.8	25.7
Sep. 1, „	H.	68	66	60	58	58	60	60	61	58	54	64	64	72
	B.													
	T.	20.2	23.0	22.8	23.6	24.2	24.8	25.2	25.0	25.0	25.2	**25.6**	25.0	24.8
„ 2, „	H.	80	73	73	70	70	71	67	67	67	64	56	56	56
	B.													
	T.	16.2	16.4	16.4	16.6	16.6	15.6	15.4	15.4	15.4	15.4	15.1	15.3	15.4
„ 3, „	H.	88	88	88	89	89	94	88	94	94	94	65	67	67
	B.													
	T.	18.6	19.8	22.0	**23.0**	19.8	20.6	20.4	20.4	19.8	20.0	19.5	19.8	20.0
„ 4, „	H.	89	80	66	60	72	76	68	68	72	72	94	94	94
	B.													
	T.	20.6	21.6	23.4	23.0	23.4	23.7	**24.6**	21.4	22.4	22.1	22.4	22.4	23.2
„ 5, „	H.	68	66	60	63	63	63	64	72	81	77	81	70	63
	B.													*17*
	T.	17.6	19.8	21.6	21.6	23.6	23.6	23.8	24.4	24.0	24.6	**25.6**	25.4	25.2
„ 6, „	H.	84	76	73	73	70	74	74	67	74	74	67	67	74
	B.													
	T.	19.8	22.4	23.0	22.8	23.2	23.6	**23.8**	23.6	23.8	23.6	23.8	23.8	23.6
„ 7, „	H.	85	77	73	81	77	78	78	74	74	74	74	74	78
	B.													
	T.	16.2	18.2	21.0	21.0	22.4	23.2	24.0	23.6	**24.2**	22.8	23.6	23.0	24.2
„ 8, „	H.	88	84	80	80	77	77	74	70	70	73	67	70	74
	B.													
	T.	19.4	19.8	21.4	20.0	20.2	20.4	20.3	20.0	20.4	20.8	20.8	21.4	21.5
„ 9, „	H.	84	85	80	85	80	76	76	76	76	77	100	68	68
	B.													
	T.	17.8	18.8	19.2	19.6	19.8	**20.2**	20.2	19.8	19.9	19.6	19.6	19.6	19.2
„ 10, „	H.	89	84	89	85	85	76	85	85	85	85	89	89	94
	B.													
	T.	19.6	20.0	20.8	21.2	21.4	23.2	24.2	25.1	25.4	25.1	25.0	25.2	25.4
„ 11, „	H.	100	100	100	95	95	90	86	86	86	86	86	86	86
	B.													
	T.	19.2	20.2	20.1	20.6	20.3	22.6	**22.7**	22.6	22.6	22.6	22.6	22.4	22.6
„ 12, „	H.	89	95	95	90	77	81	70	66	66	66	66	66	66
	B.													
	T.	15.6	16.9	19.0	19.2	19.3	19.6	20.2	20.6	20.4	20.6	**21.0**	20.9	21.0
„ 13, „	H.	88	83	84	80	75	72	72	72	72	72	68	72	68
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
23.8 76	23.6 72	23.4 69	22.8 65	22.8 66 *50*	23.0 62 *48*	22.4 63 *3*	23.1 60	22.8 60	22.2 59	21.8 60	21.8 58 **3**	21.3 60	21.4 59	20.6 62	20.0 63	19.8 63	
25.0 68	24.8 68	24.2 66 *3*	24.4 65	24.2 68	23.2 67 *7*	22.8 68 *10*	23.4 84 *8*	22.8 84 *12*	22.0 89	21.8 84	21.4 84	21.2 79	21.2 79				
25.0 54	24.4 61	24.6 62	23.6 62 *1*	24.0 67	23.4 63	23.2 66 *4*	22.8 66 *2*	22.8 66 *8*	22.2 66 *4*	22.2 69 *1*	21.7 72	21.4 72	21.0 72				
15.5 67	15.6 *70*	15.6 *67*	15.8 *74*	16.4 *74*	16.6 *73*	16.8 *73*	17.0 *77*	17.2 *73*	17.0 *77*	17.4 *81*	17.4 *80*	**17.5** *80*	17.2 *85*				
21.0 100	21.2 100	21.8 100 *12*	20.6 100 *28*	20.4 100 *8*	19.8 94 *4*	19.8 94 *6*	18.2 94 *3*	18.2 94 *3*	18.2 89	18.2 94	18.2 89	17.8 89	17.6 89				
23.4 63 *4*	23.0 63 *1*	22.6 66	24.0 58	23.2 57	23.2 57	22.2 59	21.2 62 *3*	20.8 65	20.8 58 *2*	21.2 65	21.6 59	21.4 58	21.2 58				
25.8 68	26.4 68	26.4 68	26.0 68	25.2 71	25.6 75	25.6 75	24.4 75 3	24.8 74 *3*	24.6 74	23.2 78 *5*	23.0 77 *2*	22.2 81 *1*	22.0 81				
23.4 74	23.2 73	23.2 63	22.6 73	22.6 73	22.6 73 *8*	22.8 73 *6*	22.6 73 *8*	22.2 73 *6*	22.0 73 *4*	21.8 77 *2*	21.2 76	20.8 80	21.0 80				
23.0 73	23.7 78	22.2 73	23.0 77	24.2 70	23.8 74	23.2 74	23.4 77 7	22.8 77 *4*	22.2 81 *4*	22.0 81	21.4 85	20.8 85	20.2 90				
21.6 66	**22.0** 66	21.6 69	21.4 72	21.2 80 *11*	21.4 68 *3*	21.4 68 *1*	21.4 68 *1*	21.2 68	21.4 68	21.2 68	20.8 72	20.4 72	20.1 72				
19.2 94	19.2 94	18.8 94	18.8 94	18.4 94	18.3 94	18.3 94	18.4 94	18.4 94	18.4 94	18.2 94	18.2 94	18.2 94	18.2 94				
26.8 79	27.0 79	**27.2** 72	26.2 75	25.0 74 *36*	24.4 74 *35*	23.8 74 *11*	23.5 78 *13*	23.6 78 7	23.2 77 *3*	22.6 86	22.3 86 *2*	22.0 86	21.8 86				
22.7 66	22.6 66	22.7 66	22.4 69	21.8 69 *19*	21.5 68 *10*	21.0 72 *16*	20.8 72 *5*	20.6 72	20.8 72	21.0 65	20.2 72	19.6 76	19.2 75				
21.0 68	20.5 68	20.6 72	20.5 68	20.0 68	20.0 72	19.9 72 *5*	19.7 72 *4*	19.7 76 *1*	19.8 72 *1*	19.6 72	19.4 75 *1*	19.3 71	19.2 75				

APPENDED

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		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Sep. 11, 1927	T.	19.6	20.0	20.8	21.2	21.4	23.2	24.2	25.1	25.4	25.1	25.0	25.2	25.4
	H.	100	100	100	95	95	90	86	86	86	86	86	86	86
	B.													
„ 12, „	T.	19.2	20.2	20.1	20.6	22.3	22.6	**22.7**	22.6	22.6	22.6	22.6	22.4	22.6
	H.	89	95	95	90	77	81	70	66	66	66	66	66	66
	B.													
„ 13, „	T.	15.6	16.9	19.0	19.2	19.3	19.6	20.2	20.6	20.4	20.6	**21.0**	20.9	21.0
	H.	88	83	84	80	75	72	72	72	72	72	68	72	68
	B.													
„ 14, „	T.	15.6	17.8	17.8	17.8	19.0	20.0	19.8	20.4	20.4	20.4	20.6	21.0	20.7
	H.	94	84	89	89	80	76	72	76	76	72	68	65	65
	B.													
„ 15, „	T.	19.8	21.0	22.5	22.7	22.9	23.4	**23.8**	23.6	23.4	23.8	23.2	23.4	23.0
	H.	85	80	77	77	70	70	74	70	70	70	73	73	66
	B.													
„ 16, „	T.	18.8	20.0	22.0	22.2	21.6	21.4	22.2	22.0	22.9	23.9	24.0	**24.2**	23.5
	H.	89	80	73	69	81	76	81	81	77	74	70	74	70
	B.													
„ 17, „	T.	18.4	17.5	17.8	18.9	18.4	18.7	18.4	18.6	18.6	18.5	18.5	19.0	19.4
	H.	84	94	94	89	94	89	89	89	89	84	79	80	80
	B.													
„ 18, „	T.	14.3	16.8	18.8	19.8	**20.3**	19.8	19.2	19.2	19.8	19.4	19.4	19.2	19.2
	H.	94	89	89	85	85	80	89	89	85	89	89	89	89
	B.													
„ 19, „	T.	18.8	21.4	22.0	21.4	21.2	19.0	19.0	20.2	20.2	20.8	21.2	**22.2**	21.2
	H.	89	80	81	80	80	89	94	84	85	85	85	81	85
	B.													
„ 20, „	T.	16.8	18.2	19.6	20.6	20.8	22.2	21.8	21.2	21.4	22.0	**23.2**	23.0	23.2
	H.	89	70	72	62	65	59	59	62	68	59	60	51	49
	B.													
„ 21, „	T.	16.2	17.8	19.3	**20.0**	19.7	19.2	18.8	18.4	19.6	19.3	18.2	17.8	18.2
	H.	69	63	60	58	61	63	67	63	68	67	70	67	67
	B.													
„ 22, „	T.	13.4	15.4	17.1	17.8	18.7	**19.6**	19.2	18.5	18.8	19.2	19.3	19.2	18.8
	H.	82	78	79	75	67	58	67	67	70	70	67	70	70
	B.													
„ 23, „	T.	11.6	13.4	16.8	17.2	17.4	18.8	19.0	19.2	19.6	19.8	19.6	20.2	20.2
	H.	87	87	79	70	74	71	71	67	68	68	68	68	64
	B.													
„ 24, „	T.	12.2	14.8	16.8	18.2	18.6	19.8	19.8	20.4	21.2	20.0	20.2	**20.6**	20.4
	H.	87	73	66	67	67	68	64	61	55	61	61	65	64
	B.													

TABLE 3.

mental-plants 30)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
26.8 79	27.0 79	**27.2** 72	26.2 72	25.0 75	24.4 74	23.8 74 2	23.5 74 2	23.6 78 8	23.2 78 1	22.6 77	22.3 86 1	22.0 86	21.8 86				
22.7 66	22.6 66	22.7 66	22.4 69	21.8 69 1	21.5 68 6	21.0 72 38	20.8 72	20.6 72	20.8 72	21.0 65	20.2 72	19.6 76	19.2 75				
21.0 68	20.5 68	20.6 72	20.5 69	20.2 68	20.0 72 3	19.9 72 18	19.7 72 5	19.7 76	19.8 72 2	19.6 72	19.4 75	19.3 71	19.2 75				
21.0 68	**21.2** 65	21.2 65	20.8 65	20.8 65	20.8 75	20.6 68 2	20.5 68 32	20.4 68 9	20.4 72 3	20.4 68	20.2 72	19.9 76	19.6 80				
22.6 73	22.1 77	22.4 77	23.0 73	22.2 77 136	22.0 77 26	22.0 77 4	21.8 77 3	21.8 81	21.3 76	21.3 80	21.2 80	21.0 80	20.8 85				
23.2 73	22.4 73	22.0 73 33	21.8 77 20	21.6 73 6	22.0 73 23	22.7 63	22.8 63	21.6 69	21.3 68	21.2 65	20.8 65	20.0 72	19.4 80				
19.5 75	**20.0** 76	20.0 80	19.9 68	19.3 80	19.2 80	18.7 75	18.2 79	17.9 79	17.4 83	17.0 83	16.9 83	16.7 83	16.3 83				
19.0 83	19.0 83	19.2 83	19.8 85	20.0 85 56	20.0 85 9	20.2 85 44	19.0 94 23	19.2 89 18	19.2 84 8	19.0 89	18.6 89	18.6 89	18.4 90				
20.4 80	20.6 80 68	20.3 89 6	20.3 80 55	20.0 85 36	21.0 80 18	20.0 85 3	19.6 85	20.4 80	20.0 80	19.0 89	18.5 79	18.2 84	17.8 79				
23.1 49	22.8 47	22.8 54	23.0 54	22.2 51	22.2 53 25	22.3 51 13	22.3 48 16	21.8 51 25	21.4 53 23	20.8 53 12	20.7 58	20.4 68	20.2 61				
18.1 67	17.2 79	17.1 74	16.8 74	16.2 78 1	15.2 83	14.2 88	13.4 94	13.3 94	13.2 94	13.3 87	12.9 94	12.8 94	12.6 94				
19.4 70	19.4 70	18.6 70	18.2 70 31	18.4 70 31	18.2 70 21	17.6 75 20	17.6 75 13	17.4 74 8	17.2 74 3	17.8 84	17.7 75	18.0 79	17.6 75				
20.4 65	**20.8** 65	20.6 64	20.6 64	20.4 68	20.2 68	20.0 64	19.6 72 1	19.3 75 14	19.2 71 12	19.4 71 7	19.4 71 3	19.0 75	18.8 80				
20.4 64	20.4 64	20.2 64	20.2 68	19.8 64	19.6 61 10	19.4 63 20	19.6 64 28	19.0 68 17	19.0 67 2	18.8 71	18.4 71	18.0 75	17.8 75				

TABLE 3—

		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Sep. 25, 1927	T.	11.6	14.4	15.8	17.0	18.6	18.6	19.2	18.2	18.6	18.8	19.0	19.2	19.2
	H.	87	72	69	74	75	71	71	67	67	63	69	71	67
	B.													
„ 26, „	T.	12.4	14.6	15.9	17.1	18.0	18.0	18.4	18.7	19.0	19.4	19.7	19.9	20.0
	H.	87	78	78	74	75	70	63	67	67	71	71	71	64
	B.													
„ 27, „	T.	16.6	18.8	20.4	20.2	20.8	20.6	21.2	21.2	21.2	20.8	20.6	21.2	**21.4**
	H.	78	75	64	64	65	65	62	58	58	62	62	65	58
	B.													
„ 28, „	T.	15.8	19.0	20.6	21.2	21.3	22.2	22.6	22.6	22.6	21.8	22.0	22.3	22.6
	H.	94	67	68	65	62	62	66	63	63	62	66	66	63
	B.													
„ 29, „	T.	15.5	18.8	20.8	20.8	21.0	21.4	21.6	21.6	21.4	**21.8**	21.8	21.4	21.4
	H.	78	80	68	68	65	62	59	59	58	62	59	65	62
	B.													
„ 30, „	T.	14.2	15.6	17.8	19.2	19.8	19.8	20.8	21.2	21.8	21.6	21.6	21.6	**22.0**
	H.	94	94	84	75	80	80	72	72	69	69	69	73	66
	B.													
Oct. 1, 1927	T.	16.4	18.4	20.8	21.2	21.8	22.0	22.2	22.7	**23.0**	22.8	22.2	23.0	22.6
	H.	88	84	76	72	73	73	73	73	73	73	77	73	73
	B.													
„ 2, „	T.	18.6	19.0	18.6	18.8	18.6	18.8	19.2	19.2	18.6	20.0	**20.2**	20.0	20.2
	H.	80	80	84	84	84	80	80	80	80	80	80	81	77
	B.													
„ 3, „	T.	16.8	18.6	20.6	19.6	20.0	20.2	19.4	19.4	20.2	20.6	21.2	21.6	**22.0**
	H.	94	84	76	79	76	76	89	80	89	85	80	81	77
	B.													
„ 4, „	T.	16.8	18.0	21.0	21.6	20.8	19.8	21.3	21.4	21.8	21.4	21.1	21.7	**22.4**
	H.	88	72	65	66	65	59	58	65	66	68	68	66	62
	B.													
„ 5, „	T.	14.4	18.0	21.0	21.6	20.8	19.8	19.6	19.8	20.8	21.2	21.4	20.6	21.0
	H.	88	70	76	69	72	76	76	80	72	72	72	76	76
	B.													
„ 6, „	T.	14.2	17.4	21.0	21.4	22.4	22.6	22.3	22.2	22.4	22.0	22.4	22.6	22.8
	H.	63	62	58	58	59	60	59	62	59	66	66	66	66
	B.													
„ 7, „	T.	15.2	18.4	21.0	21.1	21.4	22.2	21.6	22.8	23.3	23.2	23.6	23.2	23.0
	H.	94	89	76	72	76	72	80	73	73	73	70	73	73
	B.													
„ 8, „	T.	16.0	19.0	21.0	21.0	21.6	21.6	**21.8**	21.2	21.6	21.4	21.4	21.2	21.4
	H.	100	71	65	65	66	62	62	65	66	65	65	65	65
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
19.4 71	19.0 71	19.0 71	18.8 71	19.2 71	18.8 71	18.8 17 *2*	18.6 71 *6*	18.4 71 *5*	18.2 71 *10*	17.4 70 *7*	16.8 74	16.4 79	16.6 79				
20.2 68	**21.0** 68	20.0 72	20.2 64	20.0 68	19.9 68	20.0 80	20.6 76 *7*	19.6 76 *60*	19.6 72 *22*	19.4 75 *12*	19.6 80 *4*	18.8 84	18.0 89				
20.2 61	21.2 58	21.4 58	21.2 58	21.1 62 *16*	20.4 68 *24*	20.2 64 *36*	20.2 64 *15*	19.8 64 *5*	19.6 68	19.0 63	18.4 75	18.2 75	18.6 70				
23.2 66	23.0 66	**23.4** 66	22.6 66	22.4 66	21.6 66	21.6 69 *3*	21.6 37 *15*	21.2 72 *24*	21.0 68 *10*	20.2 76 *16*	19.6 80	19.4 84	19.0 84				
20.8 68	20.4 72	20.6 68	20.8 65	20.2 65 *1*	20.2 68 *9*	19.8 76 *7*	19.6 68 *10*	19.6 68 *16*	19.2 71 *7*	19.0 71 *3*	18.8 71	18.6 75	18.2 80				
21.8 67	21.7 69	21.8 73	21.4 72	21.0 76	20.4 80	20.0 80 *12*	19.7 76 *2*	19.3 80 *4*	19.0 80 *7*	18.4 84 *6*	18.2 84	18.0 84	17.6 89				
22.4 73	22.6 73	22.4 73	22.0 77	21.8 80	21.9 77 *31*	21.2 77 *27*	21.6 77 *27*	21.0 85 *20*	20.6 80 *6*	20.6 80	20.2 85	20.0 85	— —				
19.8 76	19.8 76	19.6 76	19.6 76	19.4 80	19.4 80	19.0 80	18.6 80	18.2 80	18.0 80	17.8 80	17.6 94	17.4 94	— —				
20.8 76	20.8 76 *33*	20.8 76 *24*	20.8 76 *8*	20.4 80 *24*	20.2 80 *19*	20.2 80 *10*	20.2 80	20.2 80	19.8 80	19.6 84	19.2 89	— —	— —				
22.2 62	21.6 62 *4*	21.0 65 *15*	20.6 65 *21*	20.6 72 *31*	20.2 72 *28*	19.6 68 *10*	19.4 71 *3*	19.4 75 *2*	19.0 67	18.4 70	18.0 79	17.6 79	— —				
21.4 76	**21.8** 59	21.6 59	21.4 53	21.4 55	21.2 55 *2*	20.0 61 *21*	20.8 55 *19*	20.6 58 *17*	20.4 58 *20*	19.8 64	19.2 61	— —	— —				
23.0 66	22.4 66	22.2 69	21.4 72	20.6 80	20.2 85 27	20.0 85 *14*	20.5 80 *2*	21.2 80	20.4 80 *30*	19.0 80 *5*	18.8 80 *8*	18.6 84	— —				
22.8 77	22.9 77	2.30 73	22.2 81	**23.7** 78	23.4 73 *5*	22.0 81 *12*	21.2 85 *26*	20.8 90 *17*	20.[illegible] 89 *2*	20.2 80	19.8 89	19.6 89	— —				
21.8 66	21.4 65	20.8 68	20.6 68	20.5 72	20.0 72 *27*	19.6 72 *18*	19.4 71 *11*	19.2 75 *3*	19.0 75	18.8 80	18.6 80	— —	— —				

Table 3—

		8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.15 p.m.	12.30 p.m.	12.45 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
	T.	15.0	14.9	15.8	16.4	16.8	17.6	18.0	18.0	18.4	18.4	18.8	19.0	19.1
Oct. 9, 1927	H.	94	94	88	88	83	84	79	70	79	75	80	75	75
	B.													
	T.	15.4	18.2	18.8	19.4	**20.4**	19.6	19.6	19.4	19.5	19.4	18.2	18.0	17.6
„ 10, „	H.	73	63	67	63	64	72	64	63	60	54	59	59	56
	B.													
	T.	9.8	11.6	13.2	13.8	14.1	14.4	15.2	15.4	15.4	14.6	15.0	15.4	15.6
„ 11, „	H.	80	76	76	68	68	68	68	68	68	68	68	64	64
	B.													
	T.	7.5	6.8	9.0	9.8	11.8	11.2	**12.8**	12.2	12.8	11.8	11.8	10.4	10.0
„ 12, „	H.	78	85	74	69	70	70	76	71	69	66	76	74	74
	B.													
	T.	10.4	11.6	11.2	12.6	**13.6**	12.8	12.8	13.2	13.5	12.5	11.4	11.8	11.8
„ 13, „	H.	80	81	81	76	77	76	82	82	76	81	81	76	76
	T.													
	T.	9.6	13.2	12.6	12.6	11.2	13.6	14.6	13.8	13.0	14.7	14.8	**15.0**	14.4
„ 14, „	H.	80	71	71	76	81	77	68	68	67	64	64	64	63
	B.													
	T.	11.0	11.4	13.4	14.2	**14.8**	14.2	14.9	14.4	14.4	14.0	13.3	12.6	12.4
„ 15, „	H.	81	75	82	77	78	68	73	77	68	68	76	81	93
	B.													
	T.	7.4	10.6	13.8	14.6	15.4	15.8	15.4	16.2	16.2	15.8	15.6	16.0	**17.6**
„ 16, „	H.	78	87	68	73	68	65	69	69	69	74	78	78	70
	B.													
	T.	7.2	8.6	10.6	11.8	12.2	12.8	13.4	**13.6**	13.4	13.6	13.4	13.0	12.4
„ 17, „	H.	100	100	100	93	87	87	82	88	82	77	76	76	81
	B.													
	T.	9.2	12.5	14.4	14.4	15.1	15.8	16.2	16.2	16.0	16.2	16.0	16.2	16.4
„ 19, „	H.	79	81	68	68	64	67	65	61	65	58	61	65	58
	B.													
	T.	8.8	13.6	15.2	15.8	15.6	16.4	**17.0**	16.8	16.4	16.4	16.4	16.4	16.4
„ 20, „	H.	74	77	73	69	74	69	70	66	65	65	69	74	74
	B.												**2**	**2**

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
19.2 75	19.1 75	**19.5** 75	19.3 75	19.2 75	18.8 80	19.0 80	19.0 84	18.8 89	18.4 84	18.0 84	17.4 89	— —					
17.2 66 *9*	17.2 66 *32*	16.8 62 *24*	16.4 61 *21*	16.4 61 *9*	15.8 58 *11*	15.2 57 *3*	15.0 60	15.1 60	14.4 59	14.0 59	13.4 63	— —					
15.8 64	**16.4** 58	15.4 60	15.6 63	15.2 63	15.8 63	14.4 58	14.4 62	14.8 62	13.4 66	11.8 66	11.4 65	— —					
9.0 79	8.6 86	9.6 86	10.0 80	8.0 80	9.8 80	8.8 80	9.4 80	8.8 79	8.6 79	8.4 79	8.4 85	— —					
11.0 81	11.0 81	12.4 81	12.6 82	13.0 76	13.4 76	12.6 82	12.8 76	12.8 76	12.2 76	12.0 81	11.8 81	11.0 87					
13.4 63	14.2 63	14.0 68	14.8 60	15.2 64	15.2 64	14.8 60	14.2 59	14.0 68	13.2 63	12.0 69	11.4 65	11.2 65					
12.8 87	13.4 82	13.6 87	13.4 87	13.2 87	11.7 87	11.8 93	11.8 93	— —	— —	— —	— —	— —					
16.4 74	16.0 74	15.6 69 *1*	14.6 68	14.4 72 *1*	14.2 68	13.6 68	12.6 76	12.0 76	11.8 81	11.6 81	11.0 87	— —					
12.3 81	12.2 81	12.8 82	12.6 76	12.0 87	11.8 87	11.6 87	11.0 81	10.6 88	10.4 86	10.0 86	9.7 86	— —					
16.8 61	16.8 62	16.2 62 *1*	16.6 62 *15*	16.6 62 *74*	16.8 66 *15*	16.6 59 *16*	16.2 61 *25*	16.0 65 *15*	15.6 65 *12*	14.2 72 *5*	— —	— —					
16.6 69 *1*	16.2 69 *1*	16.0 69	16.0 78	16.0 69	16.0 78	15.9 83	16.0 88	15.4 88	15.0 88	14.8 88	14.8 88	— —					

APPENDED

(Number of experi

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Jul. 11, 1928	T.	19.5	20.5	22.0	25.8	26.8	27.3	27.4	27.5	28.1	28.1	**28.5**	27.7	26.6
	H.	95	92	87	76	68	68	68	67	62	59	59	67	70
	B.													
,, 12, ,,	T.	19.5	20.0	21.5	23.5	25.3	26.0	26.4	26.8	**27.0**	27.0	27.0	26.5	26.5
	H.	95	95	85	80	71	69	65	62	61	62	62	62	63
	B.													
,, 13, ,,	T.	16.5	17.2	19.7	21.8	24.3	25.7	20.7	27.3	28.0	28.0	27.3	27.2	27.3
	H.	100	95	83	79	67	63	62	56	55	56	59	59	57
	B.													
,, 14, ,,	T.	18.8	20.5	22.8	23.1	24.0	24.5	25.5	26.0	26.2	26.5	26.3	26.2	26.5
	H.	86	82	76	73	70	71	68	66	66	67	66	66	66
	B.													
,, 15, ,,	T.	20.8	22.0	22.2	23.4	25.5	25.4	24.8	25.0	24.5	26.3	**26.8**	26.1	26.5
	H.	86	81	79	76	78	71	74	74	74	69	67	70	70
	B.													
,, 16, ,,	T.	21.0	21.4	21.5	22.0	22.2	**22.5**	22.5	22.3	22.0	22.1	22.1	22.1	22.0
	H.	97	95	95	94	94	93	92	94	97	96	96	96	96
	B.													
,, 17, ,,	T.	19.2	19.4	19.8	21.5	24.7	24.5	25.1	**25.7**	25.5	24.4	23.2	22.0	21.4
	H.	97	96	97	90	78	78	75	76	72	83	88	79	92
	B.													*260*
,, 18, ,,	T.	18.5	20.8	24.0	**25.0**	24.0	24.5	23.5	23.0	22.7	22.5	22.5	22.5	22.5
	H.	100	87	74	72	76	75	75	79	80	81	81	81	82
	B.													*5*
,, 19, ,,	T.	17.5	18.5	20.0	20.6	22.4	22.4	22.4	22.4	22.8	23.0	**23.5**	23.5	23.0
	H.	94	87	80	74	68	68	70	68	65	60	63	63	63
	B.													
,, 20, ,,	T.	18.7	19.8	21.2	22.0	23.5	23.4	23.5	24.3	24.8	25.2	25.5	26.0	26.5
	H.	87	86	74	71	67	69	68	70	66	63	63	61	61
	B.													
,, 21, ,,	T.	17.4	18.5	20.5	22.6	24.0	24.5	25.0	26.0	26.8	27.4	**27.5**	27.4	26.6
	H.	90	86	72	76	69	65	65	60	55	52	53	56	70
	B.													
,, 22, ,,	T.	15.2	16.0	20.0	23.0	25.0	25.5	26.2	27.6	27.8	28.7	28.7	29.3	29.3
	H.	99	94	80	71	65	49	62	60	56	54	52	51	51
	B.													
,, 23, ,,	T.	22.2	23.0	24.0	24.7	25.8	25.7	26.0	26.4	25.5	26.0	26.3	26.6	26.6
	H.	75	77	70	70	64	62	61	60	63	60	57	57	57
	B.													
,, 24, ,,	T.	20.5	21.0	21.8	23.0	23.0	23.4	25.1	25.0	23.7	25.4	25.0	**25.7**	25.4
	H.	88	87	82	76	76	74	72	73	76	70	63	68	70
	B.													

TABLE 4

mental plants 15)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
25.4 71	25.0 74 *1*	25.0 73	24.6 74 *1*	23.8 79	23.3 83 *1*	23.0 80 *1*	23.1 81	22.8 81 *1*	22.7 80	— —	22.5 81	— —	22.8 81	— —	— —	— —	— —
26.4 63	26.0 63	26.0 62	25.3 66	25.0 67	24.6 74 *3*	24.1 73	23.4 76 *24*	22.5 81 *5*	22.0 82 *10*	20.7 87 *2*	20.7 88	— —	20.2 87	— —	— —	— —	— —
28.0 54	28.0 56	28.3 52	**28.4** 52	28.0 56	27.5 56	27.8 52	27.4 54	27.2 59	27.5 57 *1*	27.2 60 *3*	27.5 57 *16*	27.5 58 *19*	26.7 62 *31*	25.7 63 *25*	25.3 63	24.5 73	— —
26.7 66	26.4 67	26.5 66	27.0 67	27.0 65	27.0 66	**27.6** 65	27.2 66	27.5 65	27.0 65	26.5 67 *65*	26.3 68 *140*	26.4 67 *8*	26.2 70	25.4 70	25.4 70	25.4 71	24.5 74
25.7 72	25.7 73	25.4 74	25.0 74 *3*	25.7 74 *70*	24.7 74 *81*	24.4 75 *68*	24.0 78 *69*	23.5 79 *20*	23.5 79 *5*	23.6 80	23.4 80	23.0 81	22.7 81	22.7 81	23.0 81	— —	23.5 81
22.0 96	22.3 95	21.8 96 *2*	21.8 99 *1*	22.2 98 *8*	22.3 97 *2*	22.0 97 *2*	22.2 95 *1*	22.6 97	23.6 88	24.0 84	24.0 84	24.0 83	24.1 82	24.0 82	23.4 84	22.7 87	22.5 88
21.6 84 *100*	22.5 91 *146*	20.7 93 *120*	20.4 93 *50*	19.8 95 *70*	20.0 95 *9*	20.0 93 *4*	20.0 94	20.2 93	20.0 96	20.0 95	20.2 94	20.3 93	20.3 93	20.1 94	20.0 94	20.0 95	20.0 94
22.5 79 *7*	22.3 81 *45*	21.3 83 *183*	21.1 84 *147*	20.9 82 *105*	20.5 84 *14*	20.6 83 *9*	20.5 83	20.6 83	20.5 84	20.5 82	20.4 85	20.5 82	20.4 83	20.0 84	20.0 85	19.9 85	19.7 85
23.0 61	22.5 65 *23*	22.7 69 *68*	22.5 66 *72*	22.7 66 *108*	22.5 66 *70*	22.2 66 *70*	22.0 70 *57*	22.1 71 *27*	21.9 72 *7*	21.5 71 *4*	21.4 76	21.4 76	21.2 76	20.7 78	20.5 80	20.7 82	19.7 82
25.8 61	26.4 60	26.7 61	**27.0** 58	26.7 67	26.7 66 *2*	26.3 65 *10*	25.8 68 *19*	25.3 71 *37*	24.7 72 *168*	24.7 73 *123*	24.5 73 *135*	24.3 72 *51*	24.0 73 *13*	24.3 72 *6*	23.5 76	23.2 75	23.2 75
26.3 70	25.8 72	25.5 72	25.2 70	25.7 72	25.5 71 *3*	26.0 72 *5*	26.0 72 *12*	25.9 70 *21*	26.0 70 *46*	25.5 71 *40*	25.1 72 *115*	25.3 72 *70*	24.7 75 *60*	25.0 73 *20*	24.6 74	24.6 76	24.0 77
29.3 51	29.3 52	29.4 47	**29.5** 47	29.2 46	29.0 46	29.0 47	29.3 47	29.2 47	29.2 49	29.2 47	29.2 48	28.7 52 *20*	28.3 52 *45*	27.6 56 *55*	27.5 56 *53*	27.0 58 *71*	26.5 58 *10*
26.4 57	26.1 56	26.5 56	**27.2** 55	26.5 55	26.5 55 *9*	26.5 56 *4*	26.4 56 *51*	26.2 56 *56*	26.5 56 *68*	26.3 58 *72*	26.0 58 *26*	25.0 60 *14*	25.0 60	24.8 62	24.5 62	25.4 62	25.0 68
24.6 71	25.0 63	25.0 69	24.9 69 *16*	25.0 68 *35*	25.0 69 *17*	25.0 67 *36*	24.5 60 *33*	24.7 69 *8*	24.7 70 *4*	25.0 65	25.2 68	25.0 69	25.5 72	24.2 74	23.5 77	23.0 86	22.5 80

TABLE 4—

		6.30 p.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 p.m.	11.30 p.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
	T.	21.7	22.5	23.4	23.5	24.2	24.0	24.7	25.0	24.7	25.7	25.5	26.0	**27.0**
Jul. 25, 1928	H.	76	78	72	71	69	69	69	65	68	62	66	64	59
	B.													
	T.	19.1	20.5	23.5	26.2	28.2	28.2	28.6	28.0	29.0	29.7	29.7	30.5	30.5
„ 26, „	H.	89	77	77	60	50	50	56	56	47	44	46	44	42
	B.													
	T.	23.0	24.0	25.7	26.5	26.8	27.0	27.3	28.0	28.0	27.5	28.0	27.8	28.4
„ 27, „	H.	80	70	69	66	68	68	66	64	65	63	62	61	60
	B.													
	T.	23.5	24.0	25.5	26.6	27.7	27.5	28.0	28.0	28.4	28.5	28.3	28.5	**29.0**
„ 28, „	H.	78	77	71	69	66	67	65	64	61	61	62	60	59
	B.													
	T.	22.8	23.9	24.9	25.5	25.4	26.0	26.7	27.2	**27.6**	27.6	27.0	27.0	27.5
„ 29, „	H.	80	82	72	71	71	68	65	65	63	66	66	66	65
	B.													
	T.	22.6	23.0	23.5	24.4	24.5	25.0	25.4	25.5	25.5	**25.7**	25.0	25.0	24.9
„ 30, „	H.	84	80	81	76	73	69	70	69	69	71	73	73	74
	B.													
	T.	22.0	22.5	24.3	25.3	25.9	26.0	26.5	27.2	27.4	**27.6**	26.3	26.0	26.0
„ 31, „	H.	84	80	73	67	64	63	59	55	60	56	58	58	58
	B.													
	T.	20.0	22.4	24.0	25.5	27.7	27.0	27.7	27.7	27.3	28.0	28.8	29.3	**29.8**
Aug. 1, „	H.	89	73	65	57	50	50	49	46	48	46	42	41	38
	B.													
	T.	20.0	22.0	24.0	25.5	26.4	26.5	26.4	27.2	26.9	27.8	27.7	27.7	28.0
„ 2, „	H.	84	78	63	55	55	52	52	51	48	45	45	45	47
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 n.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
26.5 60	26.2 64	26.2 63 *2*	25.9 63 *1*	26.0 63 *3*	25.7 65 *6*	25.5 66 *10*	25.3 66 *20*	25.5 64 *20*	25.6 66 *16*	25.7 64 *8*	25.3 64	25.0 65	24.8 66	24.6 67	24.0 69	23.9 70	23.2 73
30.2 44	**31.2** 40	30.5 42	30.0 43	30.2 44	29.7 46	30.0 46	29.7 47	29.4 49	29.0 48	29.2 50	26.0 66 *45*	25.5 69 *32*	24.6 72 *19*	24.3 74 *2*	23.8 75	23.8 75	23.7 76
28.4 60	28.2 63	28.4 61	28.1 62	28.5 59	**28.8** 60	28.2 60	28.4 60	27.8 63	27.7 64 *65*	28.0 61 *15*	26.9 65 *18*	26.6 66 *14*	26.7 67 *4*	26.4 68	25.7 73 *1*	24.9 76	24.4 78
28.5 59	28.5 60	28.5 60	28.0 60	28.0 60	27.5 62	27.2 62 *34*	27.5 63 *20*	28.0 64 *7*	28.2 61 *1*	27.5 63 *3*	27.8 61 *2*	27.0 65 *3*	27.0 65 *18*	27.0 65 *6*	26.4 68 *6*	25.8 70	25.0 71
27.5 65	26.5 66	27.1 66	27.3 64	27.3 64	27.0 65	27.2 65	26.6 66 *8*	26.1 67 *34*	26.0 67 *27*	26.0 67 *26*	25.5 68 *18*	25.5 69 *3*	25.2 71 *2*	24.5 74	24.4 74	24.0 78	24.0 77
25.0 73 *3*	25.1 73 *8*	25.8 70 *14*	25.8 72 *4*	25.4 72	25.5 74 *17*	25.4 74 *12*	25.5 75 *1*	25.0 75 *10*	25.5 73 *6*	26.0 71 *2*	25.5 74	24.8 74	24.5 77	24.3 77	24.0 79	23.9 78	23.8 79
26.0 58	25.4 58	25.5 58 *2*	25.5 56 *3*	26.5 54 *1*	26.5 53 *1*	25.5 55 *13*	25.3 56 *3*	25.4 58 *4*	24.7 61	25.0 61	24.7 63	24.5 64	24.0 66	23.8 67	23.7 69	23.5 68	23.0 71
29.5 40	29.0 43	29.0 43	28.8 44	28.5 43	28.5 43	28.5 46	28.5 46	28.5 46	28.2 46	28.1 46 *2*	27.0 49 *3*	26.4 50 *7*	26.2 51 *3*	26.1 52	25.5 49	25.0 55	24.5 60
28.5 46	28.5 46	28.3 46	28.0 46	28.0 47	27.8 48	27.6 48	26.8 51	26.5 53	26.0 52 *2*	26.0 52 *1*	25.2 54	25.0 55	24.4 55	24.0 57	— —	23.6 58	23.5 59

APPENDED

(Number of experi

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Aug. 11, 1926	T.	20.5	21.0	23.7	25.5	26.8	26.6	27.0	27.2	**27.7**	27.7	27.6	27.5	27.6
	H.	89	87	79	78	65	66	64	64	62	62	62	63	62
	B.													
„ 12, „	T.	20.0	20.5	24.0	24.6	26.7	27.5	27.7	27.3	**28.0**	27.5	27.3	27.5	27.5
	H.	79	84	64	68	64	57	57	59	56	57	58	58	60
	B.													
„ 13, „	T.	23.0	24.0	25.0	25.3	25.5	26.4	**26.5**	26.5	25.5	25.0	25.4	25.5	25.6
	H.	76	68	69	67	66	63	60	63	69	71	68	69	68
	B.													
„ 14, „	T.	20.0	21.4	23.0	24.5	24.8	24.5	25.0	**26.0**	26.0	26.0	26.0	26.0	26.0
	H.	—	86	72	61	63	65	60	56	58	58	58	58	58
	B.													
„ 16, „	T.	23.5	24.0	25.4	26.1	26.5	27.0	27.2	27.6	28.0	27.6	27.5	**28.6**	28.0
	H.	81	79	72	67	64	63	63	65	62	63	63	60	61
	B.													
„ 17, „	T.	22.5	23.0	24.6	26.0	26.8	25.5	27.2	27.0	27.6	28 0	**28.4**	28.0	27.4
	H.	80	79	72	67	65	66	64	64	66	64	61	64	65
	B.													
„ 18, „	T.	24.5	25.4	26.5	26.2	26.2	27.8	28.7	29.8	29.3	29.3	**30.2**	29.3	29.0
	H.	77	74	70	72	75	65	63	57	58	59	55	58	59
	B.													
„ 19, „	T.	24.5	24.8	26.2	27.3	27.8	28.5	28.6	28.8	28.8	28.8	29.5	29.6	29.8
	H.	71	68	61	66	58	57	56	54	53	53	50	50	48
	B.													
„ 20, „	T.	23.0	26.0	28.8	29.5	31.5	32.0	32.0	32.0	**32.8**	30.4	30.8	31.0	32.0
	H.	74	64	53	55	48	49	47	49	47	53	52	51	49
	B.													
„ 21, „	T.	23.5	23.8	26.5	28.2	29.4	29.8	30.1	**30.5**	30.0	28 8	28.4	28.4	28.1
	H.	81	81	71	65	60	59	57	57	57	60	63	64	73
	B.													
„ 22, „	T.	23.2	24.4	25.8	27.0	27.5	**28.5**	28.4	28.1	27.9	28.5	28.3	28.4	28.0
	H.	93	87	79	70	65	60	63	65	64	64	65	64	65
	B.													
„ 23, „	T.	20.0	21.2	24.2	25.5	25.7	26.7	25.5	26.2	26.0	**27.4**	26.9	26.9	25.3
	H.	89	83	71	66	68	56	69	62	64	59	60	63	68
	B.													
„ 24, „	T.	22.0	22.7	24.0	23.2	24.4	25.0	25.7	**26.0**	25.5	25.3	25.5	25.4	25.0
	H.	90	87	86	93	84	71	68	68	66	60	63	68	74
	B.													
„ 25, „	T.	20.8	21.8	23.5	25.4	26.7	27.4	29.0	**29.5**	29.4	29.0	27.5	26.2	26.0
	H.	97	89	82	74	72	68	63	59	59	60	70	69	73
	B.													

TABLE 5

mental plants 15)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
27.5 63	26.5 66	27.0 64	26.8 66	26.6 62	26.6 65	26.3 67	26.0 68	26.0 66 1	26.6 60	27.0 56 2	26.7 56 2	27.0 55 27	26.7 55 7	25.9 57 1	25.5 60	25.4 61	25.0 63
27.2 60	27.2 59	27.0 59	27.0 60	26.4 63	26.5 66	26.0 66	26.5 60	26.3 60	24.6 — 13	24.0 64 9	24.4 64 5	24.4 63 3	24.7 60	25.0 58	25.0 62	25.0 60	24.5 64
26.2 67	26.2 67	26.2 64	26.2 66	26.2 66	25.5 69	25.0 73 1	24.8 74 22	24.2 75 17	24.0 77 1	24.0 77	23.7 73	23.0 79	23.0 78	22.8 78	23.0 76	22.5 80	22.5 79
25.6 58	26.0 56	25.8 55	25.5 58	26.0 55	25.7 57 2	25.0 65 9	25.1 60 14	24.5 65 14	24.4 64 12	23.8 67 8	23.6 69	23.1 71	23.0 72	23.0 71	23.0 72	23.0 72	22.7 79
27.5 62	26.4 65	25.8 67	25.5 68 2	25.7 68	25.4 70 17	25.0 71 15	25.2 71 13	25.3 69 12	25.1 69 4	24.4 72	24.6 73 1	25.0 70	24.5 72	— —	— —	— —	— —
27.5 66	27.6 66	28.0 64	28.0 64	28,0 64	27.3 68	27.8 65	27.5 65	27.6 64	27.1 66 1	27.0 74 6	27.0 66 3	26.5 66 3	26.4 69 13	26.0 70 10	25.6 73 5	25.3 71	25.0 70
28.8 59	29.2 59	29.0 59	28.8 60	28.5 60	28.5 60	28.6 60	29.0 59	28.6 59	28.4 60	27.6 61	27.5 63 1	27.1 62 3	27.0 63 12	26.7 65 19	26.6 66 14	26.2 67 4	25.7 68
29.5 47	29.3 50	29.0 48	29.0 48	29.1 48	29.4 47	30.0 46	**30.4** 45	30.2 45	30.2 45	30.1 46	30.0 45	29.8 46	29.4 47	29.2 47 1	29.0 48 10	28.5 51 24	28.0 51 18
31.5 49	31.8 49	31.0 53	31.1 53	30.7 53	30.7 54	30.5 53	31.7 50	31.0 52	30.1 55	29.1 60	29.3 60	28.6 63	28.5 64 1	28.5 64 16	28.5 67 32	29.2 59 18	29.3 57 3
28.0 65	28.5 64	28.5 64	28.5 64	28.4 65	27.9 68 2	27.5 69 5	27.0 71 4	26.3 76 22	26.0 78 18	25.8 82 2	25.8 82 1	25.7 83	25.8 82	25.8 83	26.0 81	26.0 82	25.7 83
28.0 67	27.4 68	27.4 68	27.5 68	26.8 70	26.5 70	26.5 68 6	26.5 66 3	26.0 69 3	25.9 70 14	25.3 73 14	25.3 72 10	25.0 73 4	25.0 73 3	24.9 72	24.5 75	24.2 76	23.6 80
26.5 61	26.0 64	25.4 66	25.7 64	26.0 63	26.0 63	25.8 63	25.5 67	25.5 68	25.4 67 2	24.5 71 14	24.1 70 16	24.5 69 8	24.1 70 4	23.8 71	23.5 69	23.4 69	23.0 73
24.5 77	24.5 80	24.3 79 3	24.0 78 1	24.2 77 3	23.8 82 4	23.6 82 1	23.5 81 1	23.6 83	23.8 79	24.0 81	24.9 70	23.8 82	23.5 82	23.6 84	23.4 75	23.2 88	23.0 90
26.1 73	26.4 71	26.0 73	26.0 70	26.5 70	26.4 73	26.1 72	25.7 75 2	25.8 76 2	25.4 76 2	25.0 77 6	24.5 77 2	24.4 76	24.5 75	24.5 73	24.0 72	24.0 77	24.0 77

TABLE 5—

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Aug. 26, 1928	T.	22.0	24.0	25.0	26.0	28.0	27.0	27.7	98.4	28.5	29.0	**29.4**	29.1	28.5
	H.	90	81	81	67	60	62	59	55	54	52	52	54	52
	B.													
„ 31, „	T.	22.5	23.0	22.5	23.0	24.0	24.0	24.2	24.0	24.0	23.9	23.8	24.2	24.2
	H.	—	—	—	—	95	96	96	98	96	96	97	96	98
	B.													
Sep. 1, „	T.	18.6	18.7	19.1	19.3	20.0	20.2	20.4	21.0	21.7	22.0	**22.1**	22.0	22.0
	H.	100	100	99	99	100	98	98	95	90	90	89	89	92
	B.													
„ 3, „	T.	22.4	22.4	25.4	26.2	27.0	26.6	**27.3**	27.0	27.0	26.6	26.4	26.3	26.5
	H.	90	90	76	73	74	77	75	75	75	76	78	78	80
	B.													
„ 4, „	T.	22.0	22.6	25.0	26.5	27.3	27.6	28.1	27.7	28.0	**28.8**	28.5	28.3	28.3
	H.	95	94	88	75	72	66	70	70	69	68	69	75	70
	B.													
„ 5, „	T.	23.5	24.0	24.8	25.9	27.2	27.2	27.4	27.8	**28.0**	27.3	27.6	27.5	27.5
	H.	82	83	82	75	68	68	65	64	65	68	68	67	70
	B.													
„ 6, „	T.	24.5	25.0	25.4	**27.2**	27.0	27.0	26.7	26.5	25.9	26.0	26.0	26.3	26.4
	H.	86	86	83	75	74	74	76	78	78	78	78	78	78
	B.													
„ 7, „	T.	21.7	21.7	22.5	26.3	27.5	28.0	28.6	29.3	29.0	29.6	30.4	**30.7**	30.4
	H.	84	85	83	70	67	71	60	57	54	52	48	50	51
	B.													
„ 8, „	T.	21.4	23.4	24.6	26.3	27.0	26.5	26.2	26.4	26.5	26.8	**27.4**	26.8	26.8
	H.	81	69	68	61	59	64	69	66	63	65	63	68	70
	B.													
„ 9, „	T.	18.8	19.2	21.5	22.7	24.5	25.3	26.3	26.0	25.9	26.0	**26.5**	26.2	26.0
	H.	91	93	90	86	75	73	68	70	70	68	69	66	68
	B.													
„ 10, „	T.	22.7	22.7	23.5	23.9	23.7	24.0	24.0	24.3	24.0	24.5	25.4	25.6	**25.7**
	H.	95	93	89	88	91	90	89	91	90	90	87	84	84
	B.													
„ 11, „	T.	19.5	20.5	23.6	23.4	25.5	**25.9**	25.5	25.0	25.0	24.5	24.0	23.9	24.0
	H.	95	89	73	73	69	62	62	65	64	69	69	72	72
	B.													
„ 12, „	T.	19.2	19.0	19.1	19.5	21.4	22.0	22.7	23.1	**23.5**	23.1	22.3	22.0	21.7
	H.	87	89	91	89	79	90	74	69	64	67	67	69	74
	B.													
„ 13, „	T.	19.0	19.2	24.0	**24.5**	24.5	24.5	24.3	23.7	23.3	22.5	22.8	23.0	22.8
	H.	100	100	90	87	86	84	81	93	93	96	97	95	97
	B.													1

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
28.5 56	28.1 59	27.8 57	27.8 60	27.7 61	27.8 60	27.0 63	26.5 66	26.5 68	26.5 70	26.6 72	26.5 70 *2*	26.6 69 *7*	26.5 70 *2*	26.5 73 *1*	26.5 70	26.0 70	26.0 70
24.3 96	24.0 97	24.0 96	24.0 98	24.0 98	23.8 99	— —	23.8 99	— —	23.9 98	— —	23.5 100	— —	23.5 99	— —	23.2 98	— —	22.5 100
22.0 92	21.6 94	21.5 94	21.3 96	21.0 97	21.0 98	— —	21.0 98	— —	20.7 100	— —	20.6 99	— —	20.5 100	— —	20.3 100	— —	— —
26.6 77	26.5 76	26.5 78	26.0 82	25.4 84 *5*	25.0 86	25.0 86 *1*	24.8 88 *1*	25.5 89 *2*	25.5 88	— —	24.0 90	— —	23.9 90	— —	23.5 92	— —	— —
27.6 74	27.2 74	27.4 70	26.9 74	26.3 82	25.0 84 *1*	26.0 83 *1*	25.5 84 *3*	25.3 86 *2*	25.3 86 *2*	25.0 86 *1*	24.5 88	— —	24.5 89	— —	24.0 90	— —	— —
27.0 70	27.0 70	26.6 73	26.5 74	26.1 74	25.5 80 *4*	25.2 81 *1*	25.0 81 *2*	24.8 83 *3*	24.5 86 *2*	24.4 85	24.4 85	— —	24.0 88	— —	24.0 88	— —	— —
26.2 76	26.0 78 *2*	26.3 75 *1*	26.6 75	26.4 77 *6*	26.4 77 *4*	26.3 78 *7*	26.5 75 *1*	26.2 76	25.6 81	— —	24.9 83	— —	24.3 85	— —	24.0 88	— —	— —
29.9 53	30.4 52	30.0 57	28.9 61	28.4 63	28.3 62	28.2 64 *2*	28.6 63 *2*	27.9 65	27.9 68 *6*	27.5 65 *2*	27.4 71	— —	26.5 76	— —	— —	— —	— —
27.4 68	28.0 72	27.0 70	26.7 69	27.0 69	27.0 70	26.4 70 *3*	26.2 72 *2*	25.4 75	25.0 81	— —	25.2 76	— —	24.7 80	— —	— —	— —	— —
26.0 68	26.0 70	25.6 71	25.5 72	25.3 75	25.3 74 *1*	25.2 71 *1*	25.3 68 *1*	25.3 67 *4*	25.0 69 *1*	25.0 69	24.5 69	— —	24.0 80	— —	23.5 75	— —	— —
25.4 83	25.5 82	25.5 83	25.5 83	25.5 83 *2*	25.5 83 *1*	25.5 84	25.4 83 *2*	25.3 85	25.4 83 *1*	25.0 87	25.0 86	— —	24.5 89	— —	— —	— —	— —
24.0 72	23.7 73	23.6 75 *4*	23.8 72	23.9 72 *1*	24.0 72 *3*	23.8 71	23.7 72	23.6 74	23.5 74	— —	23.0 76	— —	22.5 78	— —	— —	— —	— —
21.5 74	21.5 75	21.5 77	21.6 79	21.5 71	21.5 77	21.6 76	21.8 78	21.5 75	21.2 80	21.0 80	21.0 80	— —	20.6 83	— —	— —	— —	— —
22.6 99 *2*	22.5 100 *5*	22.5 100 *1*	22.5 99 *2*	22.5 99 *1*	22.5 99	— —	23.0 95	— —	23.0 95	— —		— —	22.7 83	— —	— —	— —	— —

TABLE 5—

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Sep. 14, 1928	T.	19.3	19.2	20.5	22.5	23.7	23.8	24.4	25.0	25.5	**26.0**	25.0	25.5	25.5
	H.	95	98	95	90	88	86	82	79	72	78	77	77	71
	B.													
„ 15, „	T.	18.0	18.8	21.5	23.0	23.8	23.0	24.4	24.2	24.5	24.5	25.2	25.0	25.0
	H.	94	89	81	76	74	80	74	73	73	73	74	71	73
	B.													
„ 16, „	T.	16.7	18.4	18.5	19.2	20.0	20.3	20.8	21.0	21.8	**22.0**	22.0	21.7	21.2
	H.	92	76	84	77	69	67	68	67	59	55	60	60	69
	B.													
„ 17, „	T.	—	16.6	17.5	18.0	18.4	19.0	**19.5**	19.0	19.0	19.0	19.3	18.5	18 8
	H.	—	67	66	64	61	58	57	59	57	57	58	56	58
	B.													
„ 18, „	T.	11.0	11.8	13.4	16.8	19.0	18.9	18.6	18.4	19.0	19.4	**20.2**	20.0	19.8
	H.	89	83	83	70	52	53	60	58	59	55	58	56	55
	B.													
„ 19, „	T.	15.0	15.3	16.3	**18.1**	17.2	16.3	16.4	16.0	16.0	16.2	16.0	15.5	15.5
	H.	87	85	82	70	76	88	87	88	81	81	84	88	88
	B.													
„ 20, „	T.	14.8	16.5	18.5	20.5	21.5	21.7	22.0	23.3	22.7	23.6	24.0	24.3	**24.5**
	H.	97	87	84	78	75	78	75	73	73	67	64	63	65
	B.													
„ 21, „	T.	13.2	14.3	19.0	16.8	18.5	19.5	19.0	19.0	19.5	20.2	**20.5**	20.2	20.2
	H.	97	96	74	95	89	77	74	73	70	68	66	64	65
	B.													
„ 22, „	T.	11.2	11.6	13.5	15.8	18.3	18.7	18 5	18 8	19.5	19.6	19.6	**19.8**	19.6
	H.	88	88	87	72	59	66	63	56	57	58	55	53	56
	B.													
„ 23, „	T.	11.0	11.8	12.8	13.8	14.6	15.1	15.7	16.0	**16.6**	16.3	16.2	16.0	16.0
	H.	94	89	87	90	93	94	92	92	93	91	90	87	87
	B.													
„ 24, „	T.	14.0	14.0	14.5	15.8	18.0	18.2	19.2	20.5	20.5	20.6	**21.0**	21.0	21.0
	H.	97	100	100	98	91	92	85	88	77	80	75	76	77
	B.													
„ 25, „	T.	22.0	22.2	24.0	**25.5**	19.5	21.5	22.1	21.6	22.0	22.5	22.6	22.1	20.4
	H.	80	88	82	77	100	87	83	79	76	72	75	74	76
	B.												1	1
„ 26, „	T.	10.8	11.5	13.6	14.1	15.7	17.3	**18.5**	17.2	17.2	17.4	17.5	17.5	17.5
	H.	68	64	62	66	57	56	50	54	57	54	55	54	55
	B.													
„ 27, „	T.	15.0	16.7	19.0	19.5	19.5	**20.0**	19.5	19.4	19.3	19.5	19.4	19.4	19.5
	H.	66	61	55	54	47	44	42	42	43	42	40	40	40
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
25.2 68	25.2 71	25.0 75	24.9 80	24.7 81	24.6 76	24.5 82 *1*	24.5 79	24.3 83 *2*	24.0 81 *1*	23.8 82 *1*	23.5 82 *2*	23.4 84	23.5 85				
25.3 68	25.7 70	25.5 69	25.4 70	25.0 73	24.0 77 *2*	23.5 76 *3*	23.5 76 *4*	23.4 77 *1*	23.0 80	— —	— —	— —	22.8 80				
21.0 67	20.0 75	20.6 70	20.6 70	20.0 70	19.8 70 *1*	19.6 71	19.7 71	19.2 73	19.0 74	— —	18.7 74	— —	18.4 73				
19.0 57	18.7 56	19.2 59	18.9 76	18.8 67 *3*	18.8 59	18.5 56	18.7 57	— —	18.5 59	— —	18.5 59	— —	17.6 63				
20.0 53	19.6 53	20.0 52	19 9 54 *1*	19.7 56 *12*	19.7 57 *8*	19.5 53 *4*	19.0 59 *2*	18.9 60 *1*	18.6 60 *1*	— —	18.2 67	— —	17.6 72				
15.5 88	15.5 85	15.3 87	15.2 88	15.2 88	15.4 88	15.3 90	15.3 90	15.2 92	15.2 90	15.2 89	15.1 90	— —	15.2 88				
24.3 64	24.0 64	24·1 68 *7*	24.0 67 *2*	23.0 72 *2*	22.5 70 *4*	22.5 66 *1*	22.0 71	22.1 71	22.4 68	— —	22.0 71	— —	21.0 80				
20.3 64	20.5 62	20.6 62	20.5 63	20.5 66	19.5 64	20.0 58	19.8 57	19.5 59	19.5 57	19.0 60	19.5 58	— —	17.5 68				
18.5 60	18.5 62	18.2 63	18.5 64	18.7 63	19.0 63	18.7 64	18.5 63 *1*	18.2 67	17.8 70 *1*	17.7 73	17.8 75	— —	— —				
15.7 87	15.5 90	15.5 94	15.5 90	15.5 92	15.3 90	15.3 90	15.2 92	15.6 92	14.5 90	14.2 95	14.0 96	— —	14.0 96				
20.5 80	20.5 80 *1*	20.8 77 *3*	20.0 81 *7*	20.0 81 *8*	20.0 81 *5*	19.9 82 *1*	19.6 83 *1*	19.4 85 *1*	19.3 86	19.2 87	19.4 85	— —	19.0 89				
20.6 77 *1*	19.9 76 *2*	18.5 76	18.5 75	17.7 75	17.6 61	16.8 67	16.2 65	16.7 61	16.5 64	16.0 67	15.8 67	— —	14.8 73				
17.5 55	17.5 55	17.5 55	17.5 56	17.5 58	17.5 55	17.5 54	17.5 58	17.7 54	17.5 55	17.5 55	17.4 54	— —	16.5 59				
19.3 40	19.4 41	19.2 37	19.0 37	18.7 42 *4*	18.5 43	18.3 42	18.1 42	18.0 43	17.5 47	17.3 47	17.0 49	— —	16.0 57				

TABLE 5—

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Sep. 28, 1928	T.	14.0	16.0	16.0	15.6	16.5	17.8	18.8	18.5	18.5	18.5	18.5	18.7	18.7
	H.	79	79	70	69	74	70	62	64	64	62	64	63	63
	B.													
„ 29, „	T.	16.0	16.0	17.0	19.0	19.2	21.2	**22.0**	22.0	22.0	21.0	18.7	19.0	20.0
	H.	88	90	87	69	62	58	57	58	55	56	75	72	67
	B.													
„ 30, „	T.	10.0	10.5	12.2	13.0	14.4	14.4	**14.5**	13.6	14.0	14.0	14.5	14.0	14.0
	H.	82	78	67	65	60	62	69	65	65	65	60	65	65
	B.													
Oct. 1, „	T.	8.0	8.4	12.0	13.0	14.7	15.5	15.9	16.2	16.3	16.4	**16.5**	16.2	16.3
	H.	91	94	72	76	63	55	47	50	49	46	47	47	45
	B.													
„ 2, „	T.	—	16.0	18.0	20.0	21.1	**21.5**	21.4	21.0	19.5	20.4	21.0	20.5	20.6
	H.	—	74	57	63	57	63	56	49	52	53	55	52	55
	B.													
„ 3, „	T.	—	13.4	14.5	18.8	19.2	19.3	19.3	15.7	15.5	15.8	16.5	17.5	18.5
	H.	—	81	78	58	56	63	56	42	76	69	72	68	56
	B.													
„ 4, „	T.	—	11.0	13.5	10.0	10.5	11.7	9.9	11.2	13.0	13.0	13.7	13.5	13.7
	H.	—	74	64	94	94	79	100	84	87	75	64	62	58
	B.													
„ 5, „	T.	—	8.0	9.2	12.0	14.6	14.8	15.3	15.8	15.5	16.0	**16.3**	16.3	16.0
	H.	—	59	72	63	53	52	49	50	53	51	51	50	53
	B.													

APPENDED

(*Number of experi*

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Jul. 4, 1929	T.	16.0	16.2	17.6	17.3	19.2	—	18.4	—	18.5	18.4	19.2	—	19.2
	H.	88	88	86	87	83	—	93	—	74	83	85	—	83
	B.													
„ 5, „	T.	15.0	—	15.6	15.9	16.3	16.2	16.2	16.4	16.3	**16.6**	16.0	—	16.0
	H.	80	—	98	95	94	93	90	90	88	86	89	—	90
	B.													
„ 6, „	T.	15.2	—	16.1	16.4	16.6	16.8	17.0	16.9	16.8	17.1	**17.2**	—	17.1
	H.	98	—	95	93	94	92	91	91	91	91	92	—	92
	B.													
„ 7, „	T.	15.2	15.4	16.2	16.6	16.9	**18.9**	17.8	18.6	18.6	18.5	18.7	—	17.6
	H.	98	98	90	88	94	84	91	91	78	77	90	—	82
	B.													1

(*Continued*)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
18.7 67	18.7 67	18.6 73	18.5 74	18.5 72	18.7 69	18.9 67	**19.0** 73	18.8 62	18.6 64	18.6 74	17.0 69	— —	17.0 83				
20.5 57	20.8 60	21.3 57	20.7 56	20.5 50	20.5 50	20.4 50 2	19.9 52	19.6 53	19.3 52	— —	18.5 45	— —	17.6 46				
14.3 64	14.0 65	14.5 64	14.3 64	12.5 71	13.0 67	13.7 68	14.2 64	14.0 64	14.5 60	13.5 65	12.8 68	12.5 69	12.5 69				
16.5 46	16.2 45	16.0 45	16.1 43	15.8 56	15.7 47	15.6 49	15.4 47	15.3 49	15.0 59	14.5 52	14.0 55	— —	13.0 59				
21.0 53	20.8 54	21.4 52	21.5 53	21.5 62	21.0 62	21.0 48 2	20.0 47	19.0 47 2	19.3 50	19.2 54	— —	17.5 56	— —				
19.0 56	18.7 55	18.5 59	18.0 58	18.4 61	18.0 68	18.5 67	19.0 63	**19.6** 56	19.6 60	19.5 57	19.0 58	18.0 63	17.4 66				
13.8 57	**14.2** 57	13.8 57	13.4 60	14.0 60	13.8 61	12.8 59	12.6 61	13.0 59	12.5 69	— —	12.8 65	— —	11.3 78				
15.8 50	16.0 53	15.5 47	15.9 50	15.7 47	16.0 43	15.8 50	15.7 55	15.6 55	15.5 55	15.0 57	15.0 58	— —	13.7 36				

TABLE 6

mental plants 15)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
— —	19.6 85	— —	19.4 87	— —	**20.2** 81	— —	19.8 83	— —	19.6 83	— —	— —	— —	20.2 81	— —	— —	— —	19.4 85
— —	16.1 88	— —	15.9 92	— —	16.2 79	— —	16.1 81	— —	15.9 92	— —	15.8 90	— —	15.6 94	— —	15.6 93	— —	15.6 93
— —	16.8 93	— —	16.8 93	— —	16.8 93	— —	16.6 94	— —	16.6 93	— —	16.6 93	— —	16.2 93	— —	15.8 98	— —	15.8 98
17.8 80 1	17.9 79	18.0 78	— —	17.4 82	16.8 93	— —	— —	17.2 79	16.4 92	17.6 82	17.9 78	— —	17.2 83	— —	— —	— —	15.8 93

TABLE 6—

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
	T.	13.8	13.8	14.7	15.4	16.6	18.0	17.9	**19.4**	18.7	17.7	17.9	18.2	18.4
Jul. 8, 1929	H.	100	100	97	93	88	92	90	86	74	77	79	77	76
	B.													
	T.	14.6	16.4	19.6	22.0	—	21.2	22.2	**22.6**	22.5	22.0	21.0	21.6	21.4
„ 9, „	H.	100	95	94	84	—	71	71	67	65	68	70	69	68
	B.													
	T.	15.2	16.4	19.6	22.0	24.5	25.1	25.8	**26.4**	25.2	25.0	23.9	23.4	22.8
„ 10, „	H.	100	94	79	84	64	59	61	58	61	62	65	67	72
	B.													
	T.	16.2	17.8	20.3	21.8	22.1	23.0	24.6	25.3	26.2	26.1	26.9	**27.0**	26.4
„ 11, „	H.	95	87	80	78	78	75	71	68	65	66	66	63	81
	B.													
	T.	20.2	22.4	26.9	28.8	29.8	30.8	30.2	31.2	30.6	30.6	**31.8**	30.4	30.1
„ 12, „	H.	85	77	66	56	52	50	53	50	48	53	48	51	53
	B.													
	T.	20.8	22.4	25.6	26.2	26.1	25.7	26.8	28.4	28.1	28.6	**30.2**	30.0	29.3
„ 13, „	H.	91	94	79	69	72	75	72	68	69	68	57	58	59
	B.													
	T.	—	—	21.8	22.4	22.8	22.9	23.2	23.3	24.0	24.4	**24.6**	—	24.3
„ 14, „	H.	—	—	96	92	92	92	91	91	90	88	88	—	89
	B.													
	T.	20.0	21.8	24.0	27.2	28.8	28.6	29.2	29.4	29.5	30.8	30.7	30.5	31.8
„ 15, „	H.	100	88	84	75	70	69	67	66	66	59	60	65	55
	B.													
	T.	22.8	26.2	29.1	31.0	**31.4**	—	26.8	26.3	26.2	26.6	28.8	28.4	28.6
„ 16, „	H.	84	73	63	57	62	—	73	74	75	74	71	69	68
	B.													
	T.	21.8	23.4	26.8	29.8	30.9	30.4	30.0	29.3	30.2	29.5	31.0	—	31.9
„ 17, „	H.	87	86	72	59	57	59	68	70	61	62	56	—	52
	B.													
	T.	21.8	22.8	24.6	26.1	26.9	27.6	28.0	**28.2**	26.4	26.4	25.8	—	25.5
„ 18, „	H.	98	92	85	67	66	62	60	59	66	71	70	—	71
	B.													
	T.	17.8	20.4	24.2	25.8	26.8	—	27.6	—	**27.8**	27.4	27.0	26.6	26.0
„ 19, „	H.	87	81	60	63	69	—	52	—	49	55	58	56	54
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
18.6	18.8	19.0	18.7	18.5	19.1	18 4	18.6	17.8	17.9	17.9	17.4	—	17.2	—	—	—	16.3
76	74	76	75	75	72	77	76	78	71	71	81	—	80	—	—	—	76
			1	*20*	*13*		*2*	*1*									
20.6	21.0	20.8	21.2	22.0	20.7	20.6	20.6	20.1	20.3	—	20.0	—	20.0	—	—	18.0	—
72	70	72	69	58	71	71	76	76	67	—	79	—	79	—	—	87	—
		10	*18*	*36*	*29*	*6*	*7*	*12*									
22.6	22.7	22.9	23.4	22.6	22.8	21.0	21.6	22.0	22.0	22.1	21.7	—	19.4	—	—	18.8	18.6
73	71	70	69	70	66	73	74	77	72	71	72	—	80	—	—	86	88
		6	*7*	*79*	*26*	*57*	*4*										
26.6	26.4	26.5	26.1	25.9	25.9	25.6	26.4	26.4	25.3	24.9	24.6	24.4	24.5	24.4	22.9	—	21.8
64	66	68	66	67	68	70	67	68	71	72	72	72	73	72	80	—	82
					1	*2*	*1*	*23*	*91*	*34*	*11*	*7*					
29.8	29.4	29.8	29.6	30.0	29.4	29.3	29.1	29.0	28.6	28.1	28.0	27.7	27.5	27.4	27.3	27.1	26.7
53	56	55	55	55	57	59	60	64	59	46	62	65	66	65	66	68	69
					2		*1*				*66*	*77*	*59*	*74*	*16*		
28.6	28.1	28.0	27.8	28.0	28.0	28.1	28.2	28.8	28.6	28.2	28.0	27.8	27.6	27.5	27.4	27.3	27.2
53	65	64	59	64	62	65	64	63	63	64	65	65	65	65	65	67	67
						2	*1*		*4*	*2*	*6*	*79*	*120*	*68*	*42*	*8*	*4*
24.3	24.2	24.2	24.1	24.0	23.7	23.4	22.0	23.2	22.8	22.1	21.8	21.1	20.4	—	20.2	—	20.0
92	92	90	89	90	91	92	92	91	93	94	96	95	95	—	96	—	98
		5	*49*	*27*	*29*	*42*	*3*	*4*	*2*	*2*							
32.4	—	**32.6**	32.0	30.9	29.7	28.4	27.0	27.3	26 9	26.8	27.5	27.9	26.6	—	27.4	—	26.2
55	—	53	60	64	70	71	74	74	73	74	73	79	74	—	71	—	71
							128	*153*	*74*	*54*	*21*	*4*					
28.0	27.8	27.8	28.4	29.2	29.0	28.2	26.6	25.4	25.4	25.2	25.5	—	23.8	—	22.6	—	22.4
68	69	69	71	67	66	70	72	80	81	78	77	—	83	—	88	—	88
							28	*91*	*36*	*19*							
32.0	31.8	30.2	—	29.6	29 3	29.3	29.2	29.2	29.6	29.2	28.6	28.6	28.6	28.6	28.0	27.8	27.6
53	55	58	—	59	59	59	59	59	59	59	59	60	59	60	61	64	64
											32	*31*	*28*	*57*	*10*	*19*	
25.4	26.4	27.0	25.8	25.2	24.6	24.4	24.0	24.0	24.2	—	25.0	—	23.4	—	23.2	—	22.6
70	65	50	51	54	55	58	59	59	58	—	56	—	64	—	65	—	72
				49	*31*	*52*	*38*	*11*	*7*								
26.0	26.4	26.4	26.2	26.2	25.8	26.2	26.0	26.0	25.2	25.2	25.2	25.2	25.2	—	24.6	—	24.0
52	52	52	54	54	55	56	56	58	59	61	61	61	61	—	65	—	71
						10	*3*	*3*	*50*	*70*	*40*	*16*					

APPENDED

(Number of experi

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Jul. 22, 1929	T.	19.0	19.2	19.8	21.4	22.0	—	23.6	23.6	24.4	**26.4**	25.4	24.8	24.0
	H.	96	96	91	86	84	—	77	77	74	68	70	73	75
	B.													
„ 23, „	T.	20.8	22.0	22.2	**23.2**	23.0	22.0	21.6	21.0	20.6	20.4	20.4	20.4	20.4
	H.	100	94	94	90	94	98	98	98	100	100	100	100	100
	B.													23
„ 24, „	T.	19.4	19.8	20.2	21.6	23.8	24.8	25.8	26.2	26.0	26.4	26.6	**26.8**	25.8
	H.	100	98	96	90	79	80	76	73	69	75	77	77	78
	B.													
„ 25, „	T.	21.0	21.8	23.8	25.2	26.5	28.4	28.4	28.8	28.2	28.0	28 4	27.8	28.0
	H.	96	96	88	96	92	76	76	76	79	77	77	80	80
	B.													
„ 27, „	T.	—	—	—	—	29.4	—	30.2	—	30.8	—	31.4	31.8	31.8
	H.	—	—	—	—	70	—	66	—	69	—	62	62	62
	B.													
„ 28, „	T.	—	24.6	23.4	—	28.4	—	29.2	—	31.2	—	**31.8**	31.0	31.2
	H.	—	85	81	—	74	—	73	—	64	—	62	66	63
	B.													
„ 29, „	T.	22.9	23.4	—	25.6	28 6	30.4	**30.6**	30.0	30.6	—	30.2	30.4	29.6
	H.	98	88	—	82	63	54	56	59	50	—	56	58	60
	B.													
„ 30, „	T.	19.0	21.9	24.6	27.0	26.6	27.6	28.0	—	29.0	29.4	29.4	29.2	**29.6**
	H.	89	71	69	58	60	58	60	—	56	54	53	54	55
	B.													
„ 31, „	T.	23.2	24.2	28.0	31.2	**32.4**	32.2	31.6	31.8	31.4	31.0	31.0	31.4	31.4
	H.	88	88	74	54	59	60	58	61	60	63	65	62	62
	B.													
Aug. 4, „	T.	18.0	18.8	19 4	19 8	20.2	20.6	20.8	—	21.0	—	21.8	—	—
	H.	100	91	87	77	91	85	83	—	83	—	76	—	—
	B.													
„ 7, „	T.	21.2	22.8	23.4	25.0	28.2	—	29.8	—	30.4	—	31.6	31.7	**31.8**
	H.	91	90	84	83	71	—	63	—	64	—	58	57	58
	B.													
„ 8, „	T.	24.2	25.2	28 6	30.7	**31.5**	30.2	30.6	30.4	30.4	29.1	29.5	29.6	29.6
	H.	88	90	70	70	61	65	65	64	64	67	66	66	66
	B.													
„ 9, „	T.	20.2	22.0	23.8	25.4	27.4	28.4	**29.2**	28.9	29.0	—	28.5	28.3	28.2
	H.	100	94	86	81	72	67	62	64	65	—	64	63	65
	B.													
„ 10, „	T.	19.6	23.2	26.4	27.8	28.9	29.9	**30.3**	30.2	27.6	25.9	25.5	26.9	27.4
	H.	87	82	64	56	54	51	49	49	57	72	66	67	59
	B.													

TABLE 7

mental plants 10)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
23.2 79	22.8 82	22.6 82	22.5 83	22.6 82 *1*	22.6 83 *6*	22.6 84 *10*	23.2 82 *6*	23.8 81	23.8 88 *9*	23.6 81 *1*	24.2 81 *3*	25.0 74	23.0 80	— —	21.4 81	— —	22·0 86
20.4 100 *9*	20.4 100 *10*	20.4 98	20.4 100 *2*	20.0 100 *2*	20.0 100 *2*	20.0 100	19.6 100	— —	19.6 100	— —	— —	— —	— —	— —	— —	— —	19.6 100
25.8 80	26.0 82	25.8 77	26.0 83 *1*	26.2 78	26.6 75 *1*	26.9 80 *1*	26.8 78 *2*	26.2 77 *96*	25.0 83 *77*	24.2 85 *27*	23.8 84 *5*	23.4 84 *2*	22.8 88 *1*	— —	22.2 94	— —	21.8 94
29.0 76	**29.2** 73	28.8 77	28.6 77	28.0 85	27.2 92 *5*	26.0 96 *7*	24.6 96 *92*	24.2 96 *34*	24.4 95 *20*	— —	23.6 96 *8*	— —	24.0 94	— —	23.8 94	— —	23.4 94
31.6 62	31.6 60	31.4 59	**32.0** 62	31.6 63	31.4 62	31.4 65	31.0 64	31.0 65	30.8 64	31.0 64	30.4 70	30.4 66	29.8 68 *5*	28.4 73 *30*	28.4 74 *60*	28.2 73 *48*	27.8 76 *22*
30.8 67	30.8 67	30.6 66	31.4 56	31.2 57	30.4 62	30.0 64	31.4 68	31.4 56	31.4 59	30.8 67	30.8 68	30.0 68	29.6 69	29.3 72 *1*	28.8 70 *12*	28.2 76 *86*	27.2 79 *67*
29.8 59	29.8 60	29.0 62	28.8 60	28.4 62	28.4 63	28.4 60	28.2 60 *3*	28.0 64 *36*	27.8 65 *49*	27.2 66 *36*	26.8 69 *38*	27.0 69 *14*	25.8 69 *4*	— —	26.4 75	— —	26.4 73
29.2 53	29.4 53	29.4 54	29.6 61	29.9 57	29.4 54	29.5 54	29.0 56	29.2 59	29.0 59	29.0 59	29.6 59	29.4 57 *6*	29.6 59 *3*	29.0 60 *12*	28.6 59 *61*	28.2 60 *6*	27.8 65
31.4 62	31.4 63	31.0 68	30.2 69	30.0 69	29.8 69	30.0 72	30.4 66	30.8 67	29.6 73	29.4 74	29.4 73	29.8 74	29.6 70	29.2 75	29.6 73	28.8 76 *1*	29.0 75 *10*
— —	22.4 71	— —	— —	— —	23.0 72	— —	— —	— —	**23.6** 75	— —	—	— —	21.4 96	— —	— —	— —	20.6 89
31.6 58	31.5 61	31.2 60	30.6 59	31.2 58	31.0 62	30.0 63	30.4 63	30.0 63	30.4 64	30.2 64	29.8 66	29.9 64	29.6 66	29.0 67 *3*	28.8 70 *12*	28.6 71 *26*	28.4 71 *13*
29.0 62	28.2 68	26.7 75	26.8 76 *3*	24.8 85 *6*	24.4 88 *15*	24.4 87 *9*	24.0 88 *8*	24.1 87 [illegible]	23.9 85 *2*	23.6 87	23.3 89	— —	22.8 88	— —	22.8 88	— —	22.8 89
28.5 65	28.2 65	28.4 59	28.6 56	28.3 60	28.2 61	28.0 60	27.7 62	27.6 64	27.4 62	27.2 65 *5*	27.0 68 *3*	27.0 68 *10*	26.8 69 *13*	26.8 70 *6*	26.3 70 *16*	25.9 70 *4*	25.8 71
27.6 62	27.6 67	27.6 65	27.4 68	27.2 68	27.1 70	27.0 64	26.8 68	27.0 66	26.8 66	26.6 66	26.4 68 *23*	25.8 70 *26*	25.5 72 *12*	25.5 72 *3*	25.1 73 *5*	25.0 84	24.6 76

TABLE 7—

		6.30 a.m.	7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.
Aug. 11, 1929	T.	20.2	23.8	25.1	26.2	26.6	26.3	26.9	26.6	26.8	**27.0**	26.0	25.6	25.2
	H.	89	74	72	69	68	64	64	66	63	60	61	62	66
	B.													
„ 12, „	T.	18.0	21.0	22.9	23.4	25.9	26.0	26.8	26.8	26.6	26.6	26.4	—	**26.9**
	H.	89	80	75	74	62	61	62	59	59	59	58	—	58
	B.													
„ 13, „	T.	17.6	—	23.6	25.0	25.4	25.6	25.6	**26.0**	26.0	25.9	24.7	24.8	24.9
	H.	93	—	66	60	61	57	57	53	51	51	59	58	54
	B.													
„ 14, „	T.	20.2	—	22.7	23.0	23.4	23.8	23.2	—	23.8	24.8	25.2	—	25.8
	H.	83	—	72	73	74	72	77	—	72	69	68	—	67
	B.													

APPENDED

(Number of experi

		7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.	1.45 p.m.
Sep. 20, 1929	T.	14.8	17.8	19.2	20.5	20.9	21.2	21.2	21.4	21.5	22.7	**23.0**	21.8	22.0
	H.	90	76	59	59	46	40	43	40	39	40	40	37	44
	B.													
„ 21, „	T.	13.2	16.8	19.5	**22.6**	22.5	21.6	21.0	20.2	19.4	17.2	16.4	—	16.1
	H.	66	65	57	43	42	50	52	66	51	86	93	—	95
	B.													
„ 22, „	T.	8.2	14.1	15.4	15.8	16.6	17.0	17.4	18.2	18.0	18.0	18.2	**18.4**	18.4
	H.	91	55	50	51	49	46	45	46	46	45	43	45	45
	B.													
„ 23, „	T.	9.0	12.0	16.2	18.7	18.6	18.8	19.2	19.2	19.6	19.6	**19.8**	19.5	19.4
	H.	85	77	62	58	61	62	62	62	60	60	57	57	59
	B.													
„ 24, „	T.	13.8	16.4	18.8	20.0	20.3	20.5	20.2	20.2	**21.5**	21.2	—	21.0	21.0
	H.	75	67	65	61	61	63	61	61	53	51	—	52	48
	B.													
„ 25, „	T.	—	18.4	18.7	19.7	19.4	19.6	19.5	20.1	21.2	21.6	22.0	21.0	22.0
	H.	—	68	73	71	69	73	75	72	63	56	58	62	61
	B.													
„ 26, „	T.	11.6	16.0	17.2	18.8	19.2	19.2	19.8	20.2	**21.0**	21.0	20.8	20.6	19.8
	H.	86	74	71	63	62	60	62	59	56	56	53	53	53
	B.													
„ 27, „	T.	12.6	15.4	17.6	18.4	18.6	18.4	19.1	18.9	—	19.2	19.0	**19.4**	19.1
	H.	89	76	68	58	53	55	52	55	—	52	54	56	56
	B.													

(Continued)

1.45 p.m.	2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	**6.00 p.m.**
25.2 66	25.2 68	25.2 66	25.2 68	— —	24.8 69 *13*	24.2 72 *46*	24.0 72 *12*	23.8 74 *6*	25.0 70 *1*	— —	— —	— —	24.0 72	— —	— —	— —	21.8 84
26.5 58	26.0 57	25.8 58	25.9 58	25.9 59	25.9 59	25.4 61	25.0 60 *8*	24.7 61 *14*	24.6 63 *30*	24.4 63 *23*	24.1 63 *16*	24.0 63 *12*	23.8 64 *2*	— —	23.1 69	— —	22.2 70
— —	23 8 60	23.8 60	23.6 63	23.1 64 *19*	23.2 63 *7*	23.2 65 *16*	23.2 65 *7*	23.0 65 *9*	22.8 68 *4*	22.6 65 *1*	22.4 66	— —	21.6 71	— —	21.4 72	— —	21.3 71
26.2 64	25.6 64	25.8 68	25.9 63	**26.2** 67	25.3 66	25.2 71	24.2 75 *28*	24.2 75 *16*	24.3 74 *6*	24.0 77 *3*	23.9 76	— —	23.4 79	— —	— —	— —	23.0 80

TABLE 8

mental plants 15)

2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
22.0 45 *1*	22.6 41 *1*	22.4 43	22.6 39	22.0 39	22.2 39	21.8 39	21.7 40	21.6 47	— —	— —	— —	20.4 53				
15.4 93 *2*	— —	15.4 93 *3*	— —	15.4 90	15.4 86	— —	— —	15 4 88	— —	— —	— —	14.6 67				
18.4 46	18.0 47	18.2 47	17.7 47	17.8 47	17.6 49	17.6 49	17 6 49	17.6 49	— —	17.0 50	— —	16.0 55				
19.2 58	19.2 58	19.2 60	18 8 59	18.6 59 *9*	18 4 61 *5*	18.2 61 *2*	18.2 61 *3*	17.6 61	— —	17.4 51	— —	16.6 54				
20.8 53	20.8 53	20.6 55	20.6 56	20.6 57 *12*	20.4 57 *4*	20.2 57 *3*	19 7 59 *3*	19 4 60 *1*	— —	18.2 63	— —	17.8 66				
22.6 58	**24.0** 58	23.8 48	22.2 55 *10*	22.6 54	23.0 54	22.8 54 *1*	22.4 57 *1*	22.5 57	— —	21.4 59	— —	20.6 77				
20.2 55	19.2 57 *4*	19.0 56 *5*	18.8 60 *6*	— —	18.0 58 *14*	17.8 64 *1*	17.6 68 *2*	— —	— —	- —	16.2 77	— —				
18.8 55	18.8 55 *1*	18.7 55 *1*	18 5 54	18.4 50 *17*	18.2 50 *4*	18.0 54 *2*	17.8 55	18.7 37	— —	— —	— —	— —				

TABLE 8—

		7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.	1.45 p.m.
Sep. 28, 1929	T.	14.0	18.4	20.1	21.2	21.8	**22.6**	22.0	22.2	21.7	21.8	21.8	21.8	21.8
	H.	87	67	63	54	47	51	53	57	59	57	59	59	59
	B.													
„ 29, „	T.	15.0	17.6	19.2	20.2	20.6	20.8	21.2	**21.4**	19.8	19.6	19.8	19.3	18.6
	H.	90	88	73	70	68	68	69	67	79	79	73	79	73
	B.													
„ 30, „	T.	11.8	11.9	12.4	13.4	14.0	14.0	14.1	14.3	14.4	**15.1**	15.0	14.8	14.8
	H.	84	77	74	66	62	62	62	65	57	59	58	55	55
	B.													
Oct. 1, „	T.	8.4	10.0	12.0	14.0	14.8	—	15.8	16.2	16.0	16.3	16.6	16.6	**16.8**
	H.	88	86	77	62	59	—	57	56	51	45	47	53	56
	B.													
„ 2, „	T.	9.2	12.2	15.8	18.4	17.8	18.9	19.6	21.0	21.0	21.0	21.2	21.5	21.7
	H.	91	77	74	68	64	63	59	60	57	57	57	52	52
	B.													
„ 4, „	T.	10.9	11.8	14.1	18.4	18.8	19.6	19.7	**20.0**	20.0	19.5	19.7	19.6	19.5
	H.	94	92	87	76	74	71	75	77	73	81	79	79	79
	B.													
„ 5, „	T.	—	16.6	18.0	17.4	19.4	20.3	21.2	21.0	21.3	21.2	21.2	21.2	21.2
	H.	—	73	74	89	81	73	73	68	68	63	63	65	66
	B.													
„ 6, „	T.	13.2	15.8	15.8	17.2	17.6	18.8	18.8	20.2	**21.4**	21.2	20.2	20.5	20.6
	H.	85	72	81	80	82	74	74	73	61	76	72	72	72
	B.													
„ 7, „	T.	13.6	14.5	15.4	17.8	18.0	18.2	18.5	18.4	18.6	18.8	19.0	19.0	19.2
	H.	100	95	90	76	76	78	72	72	73	73	74	74	74
	B.													
„ 8, „	T.	16.2	16.6	16.3	17.3	17.0	17.2	17.3	17.0	—	17.8	—	**18.0**	—
	H.	100	98	98	73	76	82	84	78	—	82	—	88	—
	B.													
„ 9, „	T.	10.8	12.4	14.1	16.3	16.4	16.9	17.1	**17.9**	16.8	16.6	16.7	16.8	—
	H.	89	82	85	62	63	67	60	64	57	59	72	62	—
	B.													
„ 10, „	T.	10.0	10.6	11.0	11.4	11.8	13.0	13.5	14.0	—	14.8	**15.0**	14.8	14.8
	H.	80	78	81	81	81	70	71	69	—	61	61	61	63
	B.													
„ 11, „	T.	10.8	14.0	**15.1**	14.2	14.8	13.8	13.4	13.2	13.4	13.7	—	—	—
	H.	86	62	58	57	59	60	62	64	64	60	—	—	—
	B.													
„ 12, „	T.	10.6	11.3	10.7	12.6	12.2	12.4	—	13.6	14.2	13.9	—	14.1	—
	H.	86	84	92	82	89	82	—	80	67	75	—	71	—
	B.													

(Continued)

2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
22.0 61	22.4 58	22.4 58	22.5 58	21.6 58	21.4 59 *32*	21.3 61 *7*	21.1 62	20.9 65	— —	— —	— —	18.0 79				
18.4 74 *4*	18.0 78 *17*	17.7 78 *8*	17.5 80 *2*	17.4 80	— —	— —	16.6 88	16.2 88	— —	— —	— —	15.4 88				
14.7 57	14.8 58	14.6 57	14.8 58	14.4 57	— —	— —	— —	— —	13.4 66	12.4 65	— —	— —				
16.4 51	16.6 53	16.3 51 *1*	16.6 54	16.6 57	16.4 57 *3*	16.3 53 *1*	16.0 59 *2*	15.9 59	— —	— —	— —	14.9 84				
21.5 52	**22.1** 52	21.7 52	21.7 50	21.5 49	20.8 49	20.6 57	20.5 58	19.8 64 *27*	19.4 67 *12*	19.2 69 *2*	18.0 76	17.1 78				
19.6 79	19.7 79	19.5 81	19.8 79	19.5 83	19.2 85 *3*	18.9 87 *5*	18.8 87 *4*	18.6 89	— —	— —	— —	18.0 91				
21.5 67	**21.6** 63	21.4 65	21.2 65	21.0 66 *12*	20.8 66 *22*	20.6 68 *21*	20.5 79 *2*	20.3 79	— —	— —	— —	18.6 87				
20.8 70	20.8 70	20.0 72	19.8 69 *4*	19.8 69	— —	— —	— —	19.0 76	— —	— —	— —	18.9 76				
19.0 74	19.1 73	19.0 71	18.9 73	19.0 74	19.0 76	19.2 74	**19.3** 77	19.2 86	— —	18.6 98	— —	— —				
— —	— —	— —	— —	14.6 97	— —	13.4 98	— —	12.0 98	— —	— —	— —	— —				
17.5 53	17.2 53	17.1 53 *2*	17.1 60 *26*	17.0 62 *22*	— —	16.8 62 *14*	16.4 65 *10*	16.6 63 *10*	16.8 71 *6*	16.6 77 *2*	— —	15.2 81				
— —	14.5 67	— —	14.5 69	14.2 77	— —	13.5 79	— —	13.2 80	— —	— —	— —	— —				
12.2 69	— —	10.9 78	— —	12.0 67	— —	11.0 66	— —	8.6 71	— —	— —	— —	— —				
14.6 67	— —	14.4 65	— —	14.4 69	— —	— —	— —	12.8 72	— —	— —	— —	— —				

TABLE 8—

		7.00 a.m.	8.00 a.m.	9.00 a.m.	10.00 a.m.	10.30 a.m.	11.00 a.m.	11.30 a.m.	12.00 m.	12.30 p.m.	1.00 p.m.	1.15 p.m.	1.30 p.m.	1.45 p.m.
Oct. 13, 1929	T.	7.8	9.2	9.4	11.2	12.0	12.4	—	12.6	12.6	13.0	—	**13.2**	—
	H.	94	85	74	81	77	72	—	82	87	84	—	82	—
	B.													
„ 14, „	T.	11.8	14.0	16.0	16.5	17.0	**17.5**	16.4	16.3	17.2	17.4	—	17.4	**17.4**
	H.	81	73	71	69	68	66	69	71	71	68	—	66	**68**
	B.												*105*	*89*
„ 15, „	T.	12.4	12.9	12.7	12.7	12.6	12.0	13.8	12.6	13.6	13.6	—	**14.4**	—
	H.	74	59	68	63	74	60	68	60	60	62	—	72	—
	B.													
„ 16, „	T.	5.9	10.4	12.8	13.6	14.2	14.9	14.6	**15.0**	—	14.4	—	**14.4**	—
	H.	87	72	62	55	61	58	59	59	—	61	—	58	—
	B.													
„ 17, „	T.	8.0	8.2	8.4	9.1	—	11.4	—	18.2	18.4	18.0	—	18.4	—
	H.	*97*	*100*	*97*	*97*	—	*97*	—	*76*	*76*	78	—	78	—
	B.													
„ 18, „	T.	9.0	9.8	11.6	13.0	13.0	14.0	—	15.0	—	**15.1**	—	14.8	—
	H.	85	83	69	64	66	67	—	63	—	68	—	63	—
	B.													
„ 19, „	T.	6.3	12.6	14.5	—	**16.3**	15.8	—	15.8	—	15.8	—	16.0	—
	H.	63	63	64	—	57	59	—	57	—	57	—	57	—
	B.													

(Continued)

2.00 p.m.	2.15 p.m.	2.30 p.m.	2.45 p.m.	3.00 p.m.	3.15 p.m.	3.30 p.m.	3.45 p.m.	4.00 p.m.	4.15 p.m.	4.30 p.m.	4.45 p.m.	5.00 p.m.	5.15 p.m.	5.30 p.m.	5.45 p.m.	6.00 p.m.
13.0	—	—	—	12.2	—	—	—	11.8	—	12.0	—	—				
84	—	—	—	92	—	—	—	92	—	92	—	—				
17.2	*17.0*	*17.0*	*17.0*	*16.6*	*16.6*	*16.3*	—	*15.6*	—	*15.0*	—	*13.4*				
68	69	73	83	86	88	72	—	81	—	76	—	82				
38	*7*	*24*	*17*	*4*	*6*	*2*										
11.9	—	13.4	—	12.6	—	—	—	11.4	—	—	—	—				
85	—	57	—	59	—	—	—	81	—	—	—	—				
14.6	14.4	14.5	14.3	13.8	—	—	13.0	—	—	—	—	—				
59	59	58	59	62	—	—	65	—	—	—	—	—				
	1	*3*														
19.6	—	19.2	—	19.2	—	19.2	—	18.0	—	16.8	—	—				
64	—	60	—	60	—	60	—	59	—	63	—	—				
14.9	—	14.8	—	14.5	—	—	—	—	—	12.2	—	—				
61	—	65	—	65	—	—	—	—	—	79	—	—				
16.0	15.9	16.0	16.0	15.8	—	15.7	—	14.6	—	—	—	—				
61	71	65	64	64	—	71	—	72	—	—	—	—				
14	*3*	*7*	*15*	*9*		*3*										